

FEEDING AND ON-GROWING STRATEGIES FOR
YELLOWTAIL FLOUNDER *Limanda ferruginea* (Storer)

CENTRE FOR NEWFOUNDLAND STUDIES

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**FEEDING AND ON-GROWING STRATEGIES FOR
YELLOWTAIL FLOUNDER *Limanda ferruginea* (Storer).**

by

Daniel Lawrence Boyce

**A thesis submitted to the School of Graduate Studies in partial fulfilment of the
requirements for the degree of Master of Science (Aquaculture)**

**Ocean Sciences Centre
Memorial University of Newfoundland**

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Newfoundland

ABSTRACT

“Give a person a fish and he will have food for a day; teach him to grow fish and he will have food for a lifetime”, so goes the old Chinese saying. Application of this wisdom on a world-wide scale could go a long way in producing food for the hungry millions. To help spread this principle and philosophy is important in aquaculture. This thesis focuses on strategies to improve the growing techniques of juvenile flatfish with emphasis on yellowtail flounder, *Limanda ferruginea* (Storer), which has been identified as a potential species for cold-water aquaculture along the north-east coast of Canada.

Optimal food rations can affect the commercial success of any aquaculture venture. The effects of ration levels on growth performance of 0+ juvenile yellowtail flounder was examined for fish held under a 16L:8D photoperiod. Two experiments were conducted; the first using ration levels of 1, 2, 4, 6% (body weight·d⁻¹ (bw ·d⁻¹)) on small juveniles (mean initial weight ± SE 1.0 ± 0.04 g) held at 7.0°C with a stocking density of 0.95 kg·m⁻² (~ 45% bottom coverage). The second experiment used ration levels of 1, 1.5, 2, 3% bw ·d⁻¹ on large juveniles (mean initial weight ± SE 7.39 ± .07 g) held at 10°C with a stocking density of 1.45 kg·m⁻² (~ 34% bottom coverage). Survival in both experiments was not significantly different. Results of experiment 1 indicated that fish fed 1% bw ·d⁻¹ had significantly lower growth (weight, length, body depth and specific growth rates)(p<0.05) than those fed 2, 4 and 6% bw ·d⁻¹. Significant differences (p<0.05) with gross food conversion ratios (GFCR's) were found between fish fed rations of 1% and 2% and those

fed 4% and 6% rations, but 4-6% $\text{bw} \cdot \text{d}^{-1}$ were poor in terms of gross food conversion ratios, resulting in food wastage. Results of experiment 2 indicated that fish fed 1, 1.5 and 2% $\text{bw} \cdot \text{d}^{-1}$ had significantly lower growth (weight, length and specific growth rates) ($p < 0.05$) than fish fed 3% $\text{bw} \cdot \text{d}^{-1}$. Gross food conversion ratios (GFCR's) were significantly different ($p < 0.05$) for all 4 rations. Overall, this study demonstrated that it would be more feasible to use a ration of 2 % $\text{bw} \cdot \text{d}^{-1}$ for small juveniles and 1-1.5% $\text{bw} \cdot \text{d}^{-1}$ for large juveniles.

It has been demonstrated that increased photoperiods improve growth and survival during the larval stage of this species. I conducted an experiment to determine the effect of photoperiod on growth and survival of 1+ juvenile yellowtail flounder. This experiment compared growth and survival rates of juveniles (mean initial weight \pm SE = 9.25 ± 0.22 g) under 24, 18, 12 and daily ambient photoperiod. A stocking density of $0.47 \text{ kg} \cdot \text{m}^{-2}$ (~ 10% bottom coverage) and a feeding ration of 2% (body weight $\cdot \text{d}^{-1}$ ($\text{bw} \cdot \text{d}^{-1}$)) was used. Temperature was held at 7.0°C . No significant differences in growth or survival among juveniles were found under the different photoperiods. It appears that the most cost-effective approach is to provide a simulated natural photoperiod for juvenile yellowtail flounder.

There is a need for an “optimal stocking density” of juvenile flatfish to be established for cultured species in hatchery situations. This final study in my thesis examined the effects of three different stocking densities on the growth performance and survival of 0+ juvenile yellowtail flounder held under 16L:8D photoperiod. Three

densities of 0.47, 0.95 and 1.9 kg·m⁻² with 23%, 45% and 90% bottom coverage was used. Juvenile yellowtail (mean initial weight ± SE = 1.02 ± 0.05 g) were fed a feed ration of 2% (body weight·d⁻¹ (bw ·d⁻¹)). Temperature was 7.0°C. No significant differences in growth or survival between juveniles were found under the different stocking densities. However, stocking densities with 90% bottom coverage had slightly lower growth rates and higher gross food conversion ratios.

Overall results suggests that juvenile yellowtail flounder can be stocked at densities greater than 100% bottom coverage. Economically, it appears more feasible to use a ration of 2 % bw ·d⁻¹ for small juveniles and 1-1.5% bw ·d⁻¹ for larger juveniles and it appears that the most cost-effective approach is to provide simulated natural photoperiod (min. 6-8 hours) for juvenile yellowtail flounder production.

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CHAPTER 1

INTRODUCTION

World aquaculture production has been increasing rapidly for more than a decade, and the rate of increase has remained fairly constant. This is remarkable considering the present worldwide economic situation. Aquaculture production currently accounts for 29% of the total yield from the fisheries of the world and aquaculture is currently the only worldwide growth sector within fisheries (Anonymous, 2000). As the fishery catch is predicted to stabilize at its present level, aquaculture is considered the only option to meet the increasing demand for aquatic proteins (Harache, 1997).

In recent years, scientists have become aware that the world's oceans, lakes and rivers represent a major source of animal protein that nations are not fully utilizing. When this is coupled with the ever-expanding global human population and the finite nature of the capture fisheries, a sense of urgency is brought to bear on the development of aquaculture. Production from the capture fisheries peaked in 1989 and has since fluctuated near that level, indicating the aggregate stocks of the world are being harvested at or near their maximum sustainable yield (MSY) (Aiken & Sinclair, 1995). Many stocks are being over fished, and some have collapsed (ie. Atlantic cod). We may already be extracting the maximum from our capture fisheries, and future increases in marine protein production may have to come from the world's culture fisheries. This concept has important implications for the coastal nations of the world which may exploit aquaculture. We may however, be able to increase the productivity of the world's capture fisheries by 15-20 million tonnes annually through improved management practices (Aiken & Sinclair,

1995). However, this increase is not enough to meet the increasing demands of world's protein supply.

Aquaculture will no doubt increase its productivity in years to come. Aquaculture has the potential to become as important to the people of the 21st century as agriculture has been to those of the 20th century (Aiken & Sinclair, 1995). Fish farming will be a top performer for the new millennium and is going to be the growth industry of the next 30 years (Drucker, 1999).

Newfoundland is an ideal setting for cold water aquaculture development, possessing the right mix of abundant natural resources, technical expertise and research support. Tilseth (1990) stressed that market value, cost of production and quality of production are all important contributors to the success of marine aquaculture. For Newfoundland aquaculture, the potential species must not only be biologically suited to intensive culture, but must also be able to withstand extreme cold water conditions ($<0^{\circ}\text{C}$) which can prevail over a four to five month period (Brown *et al.*, 1992 a).

The sudden and dramatic decline in the Atlantic Canadian ground fishery (Table 1.1), has left hundreds of fishing communities in despair and caused thousands of fisher people to depend on government aid programs. This collapse of once-abundant cod and groundfish stocks is being felt throughout the region, but nowhere more deeply than in Newfoundland, where a moratorium has been imposed on both the inshore and offshore cod fisheries in most areas.

Table 1.1: Activity in the fishing industry of Newfoundland and Labrador.

	Quantity (tonnes)		% Change
	1994	1995	
Groundfish	31723	16726	-47.3
Pelagics	26798	31025	15.8
Shellfish	78463	88078	12.3
Total Landings	136984	135829	-0.8
Imports for Processing	27731	28842	4.0
Aquaculture Production	827	1280	54.8
TOTAL	165542	165951	0.2

Source, Fishery: <http://www.gov.nf.ca/exec/publicat/economy/fish.htm>

Alternative employment opportunities are few for the inhabitants of the tiny fishing communities scattered along the island's 18,000 km coastline. People whose working lives have been spent in the harvesting and processing sectors of the fishery are reluctant to leave their communities and unique way of life to seek work elsewhere.

Administrators in both the provincial and federal government are struggling to come to grips with the enormity of the problem. Training fisher people for work in aquaculture is one possible alternative for employment. Traditional fishing skills, blended with appropriate aquaculture technologies may provide a real opportunity to sustain these numerous tiny fishing communities.

Within Atlantic Canada, climatic conditions, cold ocean temperatures and winter sea ice has restricted marine aquaculture development. However, in spite of these

restrictions there are active commercial aquaculture operations: Atlantic salmon farming in the Bay of Fundy, New Brunswick, blue mussel farming along the shores of Prince Edward Island and Newfoundland, and steelhead trout farming on the south coast of Newfoundland. The fishery crises has provided impetus for a program of aquaculture development throughout the region. In Newfoundland, development agencies, fish farmers and scientists are working together to commercialize the farming of a diverse range of cold water marine species. There are many reasons for diversification within the aquaculture industry. Primary among these are to broaden the base of aquaculture in the area, to develop new products for a growing market, and to provide job opportunities for a region hard hit by the recent decline in the capture fisheries (Table 1.1 Brown *et al.*, 1995). Yellowtail flounder *Limanda ferruginea* (Storer) is being considered as a new species for marine aquaculture along with Atlantic cod, Atlantic halibut, witch flounder and spotted wolffish.

1.1 BIOLOGY AND DISTRIBUTION

Yellowtail flounder *Limanda ferruginea* (Storer) is a member of the family Pleuronectidae (Cooper & Chapleau, 1998), or right-eyed flounder, meaning that during metamorphosis the left eye migrates as the fish settles on its left side (Scott & Scott, 1988). Distribution in the northwest Atlantic extends from the Strait of Belle Isle to Chesapeake Bay including the Gulf of St. Lawrence and the Grand Banks (Pitt, 1970; Laurence & Howell, 1981) and the species is rarely found in waters deeper than 90-100 m

(Laurence & Howell, 1981; Perry & Smith, 1994). They are most frequently found at depths around 60 m at temperatures of 3-5°C. Characteristics that distinguish it from other flounder include a small mouth, concave dorsal profile of the head, a lateral line which arches above the pectoral fin, the yellow colouration of the under surface of the caudal peduncle, and the bright rust-coloured spots on the pigment side (Scott, 1947).

Yellowtail flounder produce large numbers of small pelagic eggs (750-900 μm). Eggs do not have an oil globule and hatch at approximately 60° days (Evseenko & Nevinskiy, 1982). Larvae are between 2.4-3.5 mm in standard length (notochord) at hatch. They are serial batch spawners (multiple spawnings) which spawn between May and July in Canadian waters (Zamarro, 1991). Batch spawners such as yellowtail flounder portion their vitellogenic oocyte production into a series of ovulatory events (Manning & Crim, 1998).

Yellowtail flounder became important to Canadians in the late 1960's and early 1970's when a fishery developed on the Grand Banks. The stock is mainly concentrated on the southern Grand Bank and is recruited from the Southeast shoal area nursery ground, where the juveniles and adults overlap in their distribution (Walsh, 1997). Department of Fisheries and Oceans (DFO) stock assessment data indicates that recent year classes have been poor relative to year classes in the 1970's (Walsh, 1997). This may be a result of high fishing mortality on juveniles in the late 1980's and early 1990's (Walsh, 1997). It has since been suggested that the stock should be able to sustain a limited fishery, not exceeding 4000 metric tonnes and confined to the main component of the

stock in Div. 3NO in 1998 (Walsh, 1997). Since this recent decline in the traditional fisheries, yellowtail flounder has become a species of interest for aquaculture.

1.2 REASONS TO CULTURE YELLOWTAIL FLOUNDER

Yellowtail was a commercially important species in the capture fishery until the recent decline in stocks, and there is every indication that it has good market potential (Brown *et al.*, 1995). The total allowable catch (TAC) has declined from 20,000 metric tonnes in the early 1980's to 287 metric tonnes in 1996, and as already stated the recommended TAC since 1998 is not to exceed 4000 metric tonnes in divisions 3L, 3N and 3O (Walsh, 1997).

If commercial development of yellowtail flounder aquaculture is possible, it could support a significant growth industry supplying an already developed market (Goff, 1993). Yellowtail is a medium value, small flounder with premium white flesh. Scott and Scott (1988) reported that yellowtail flounder has the fastest growth rate among the small commercial flounders. Goff (1993) reported that yellowtail flounder is the preferred choice of small commercial flatfish for aquaculture, and has the highest fillet yield of small flatfish. The fillet yield from a 30–45 cm wild yellowtail flounder can be close to 40% (Table 1.2). Fillet yield and quality may be improved when grown under ideal aquaculture conditions due to reduced foraging costs of the fish.

Table 1.2 Fillet yield from 30–45 cm. yellowtail flounder.

		30 cm / 225 g	35 cm / 375 g	40 cm / 650 g	45 cm / 950 g
<u>Month</u>	<u>% Yield</u>	<u>fillet (g)</u>	<u>fillet (g)</u>	<u>fillet (g)</u>	<u>fillet (g)</u>
January	32.5	73	122	211	309
May	34.5	78	129	224	328
July	37	83	139	240	351
October	38	86	143	247	361

Source: Goff, 1993.

Yellowtail flounder have many positive attributes from an aquaculture perspective. The flounder life cycle has been completed in culture at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland, where wild broodstock (replaced every few years) have been maintained since 1992. These wild broodstock have provided a high percentage of viable gametes, and the average fecundity per kilogram of fish can be as high as 1.5 million eggs, per fish ranging from 0.84-0.98 mm diameter (Manning and Crim, 1998). This indicates they are capable of acclimating to controlled culture conditions. Larvae reach metamorphosis relatively quickly at an age of 40-70 days at 10°C. Survival through metamorphosis to settlement has been > 50% with some egg batches at the OSC and it may be possible to consistently achieve upwards of 50% with improved protocols (refer to Appendix 1). Another positive attribute is that mortality is low after metamorphosis (<5%). At present there are F₁ broodstock held at the OSC which were reared from gametes received during the 1996 spawning season of wild broodstock.

1.3 RESEARCH TO DATE

Yellowtail flounder studies have been ongoing since the 1920's. Because of its commercial importance, most studies have dealt primarily with wild stocks, particularly distribution and stock abundance (Perley, 1852; Huntsman, 1922; Bigelow & Welsh, 1925; Hildebrand & Schroeder, 1928; Bigelow & Schroeder, 1953; Ross & Nelson, 1992; Walsh 1992), summer feeding intensity (Efanov & Vinogradow, 1973), seasonal food habits (Libey & Cole, 1979; Langton, 1983), age and growth (Pitt, 1974; Lux & Nichy, 1969), food consumption and feeding (Bigelow & Schroeder, 1953; Efanov & Vinogradow, 1973; Pitt, 1976; Collie, 1987), fecundity (Howell & Kesler, 1977), seasonal changes in ovaries of adults (Howell, 1983), reproduction (Zamarro, 1991) and diel movements of larvae (Smith *et al.*, 1978).

Research into the culture of yellowtail flounder began during the 1970's with induced spawning and larval rearing (Smigielski, 1979) and continued into the 1980's with yolk- utilization rates of yolk-sac larvae, temperature and salinity effects on egg development, growth and survival (Howell, 1980; Laurence & Howell, 1981; Howell, 1983). All published data during the 1980's that involved fecundity, egg quality and larval work pertained to the Southern New England stock.

Yellowtail flounder research began at the OSC in 1993. Wild Grand Bank yellowtail were captured by either D.F.O. trawls or OSC divers and transported to holding tanks at the OSC. These fish were maintained as broodstock, and hand stripped during the spawning season thus releasing viable gametes from which juvenile fish were produced.

This early success aided in directing research towards understanding the reproductive physiology of yellowtail flounder under captive conditions. Yellowtail research at the OSC has focussed on broodstock management, reproductive biology, egg incubation and larval rearing. Studies to date have included: neural endocrinology-physiology of reproduction (advancement of spawning using GnRH analogues, reproduction of captive yellowtail flounder, sperm quality, cryopreservation of semen), (Crim & Bettles, 1997; Larsson *et al.*, 1997; Manning & Crim, 1998; Clearwater & Crim, 1998a; Clearwater & Crim, 1998b; Clearwater & Crim, 1998c; Richardson *et al.*, 1999), growth and behaviour of larvae (French, 1995; Copeman 1996; Morris, 1997; Rabe, 1999; Rabe & Brown, 2000), larval prey densities (Puvanendran & Brown, 1995), histological and histochemical studies (Murray *et al.*, 1994a; Murray *et al.*, 1994b; Murray *et al.*, 1996; Baglole *et al.*, 1997) and lipid utilization and feeding of juveniles (Whalen, 1999).

There is little information available on optimal rations, photoperiod or stocking densities for on-growing juvenile yellowtail flounder. My research objectives are to identify rearing protocols for feeding and on-growing of juvenile yellowtail flounder. Specifically the objectives of my work were:

- 1) To determine food rations for 0+ juvenile yellowtail of different sizes.**
- 2) To determine rearing protocols for juvenile yellowtail focussing on stocking density and photoperiod requirements.**

CHAPTER 2

2.1 INTRODUCTION

“The quality and quantity (ration) of food is a prime factor in determining the growth performance of fish, but the efficiency of assimilation of available energy is markedly influenced by other factors, such as ambient temperature, stocking density, ontogeny and season” (Reddy *et al.*, 1994). Knowing the optimal ration for cultured fish is important not only in terms of fish growth, but also for economic and environmental reasons: minimising feed wastage decreases both nutritional loss and water pollution (Langar & Guillaume, 1994; Litvak, 1996). By optimizing feeding, economic benefits can also be realized. Because food ration can affect the growth of cultured fish, the commercial success of any aquaculture venture depends on knowing what the optimal food ration is. Fish are usually fed a prescribed percentage of their body weight per day (ration), *ad libitum*, or to satiation. Food ration is described as the amount of food fed per day and is expressed as a percentage of body weight per day (Quinton & Blake, 1990). The estimations of daily rations should take into account the numerous biotic (controls that are associated with the animal and their internal regulatory system) and abiotic (factors associated with the environment and quality of the diet) factors which influence appetite (Goddard, 1996; Anderson, 1999).

Food conversion ratios are important quality-performance indicators. Under routine hatchery operations low food conversions are seldom realized because there is always some food waste (Westers, 1987). Some of it simply escapes (drain) and some may be refused. Food loss can also result from characteristics of a rearing unit such as fish

rearing density, and water quality (high turbidity, for instance). All attempts should be made to minimize food loss and / or wastage.

Currently no published information is available on growth rates and optimal rations for cultured juvenile yellowtail flounder. The objective of this study was to determine the effects of ration levels ($\text{bw} \cdot \text{d}^{-1}$) on the growth performance of 0+ juvenile yellowtail flounder.

2.2 MATERIALS AND METHODS

The ration experiments were carried out at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland. Fish used in these experiments were reared from broodstock held at the OSC. Eggs were stripped from the broodstock and all larvae were reared at the OSC. Newly weaned juveniles (0+ fish) were used in these experiments. Juveniles were maintained under 18L:6D photoperiod and fed daily in large holding tanks (3000 L). Fish were then acclimated to experimental tanks for two weeks prior to the start of the experiment. Water quality parameters such as temperature, salinity, dissolved oxygen, ammonia, nitrogen and pH were monitored and were within acceptable levels during the experiment.

Water depths in raceways and tanks where flatfish are cultured is maintained at low levels compared to other species and for that reason fish density expressed as kilograms per volume unit may be as high as $800 \text{ kg} \cdot \text{m}^{-3}$ (Øiestad, 1999). However when dealing with yellowtail and bottom-dwelling or benthic fishes which lie on the bottom

most of their time when not feeding, the density should ideally be expressed as a proportion of the surface area (density per m^2) of the bottom of the experimental tank as opposed to $\text{kg}\cdot\text{m}^{-3}$ which has been adopted for salmonoids and other pelagic fish species which have a more even distribution throughout the water column. Density is expressed in $\text{kg}\cdot\text{m}^{-2}$ in this thesis (refer to Appendix 1).

Fish used in both experiment 1 and 2 were of the same age but of different sizes due to improvements in rearing protocols from one year to the next. It is important to understand that the weight/size of fish is an important factor in trying to determine the amount of food to be fed, as daily specific growth rates change from smaller fish to larger fish, along with assimilation.

2.2.1 EXPERIMENT 1

Prior to the experiment, fish were taken from holding tanks and transferred to experimental groups. Four different ration groups (with 2 replicates), of 50 fish each were established. Density (measured as percent coverage of the tank bottom) of fish in each tank at the start was $0.95 \text{ kg}\cdot\text{m}^{-2}$, which comprised ~ 45% bottom coverage (refer to Appendix 1). Fish were placed in 0.26 m diameter black circular 13.5 litre tanks (22 cm water depth), provided with filtered sea water (mean \pm SE = $7 \pm 0.3^\circ\text{C}$), at a rate of 0.5 L/min. (2.2 exchanges per hour).

Fish were acclimated to experimental tanks for two weeks prior to the start of the experiment. Fish were matched for weight (mean \pm SE = $1.00 \pm 0.04 \text{ g}$), standard length

(mean \pm SE = 3.90 \pm 0.4 cm) and body depth (mean \pm SE = 1.71 \pm 0.02 cm) at the beginning of the experiment.

Growth measurements (weight, standard length and body depth) were taken at 14 day intervals on 10 fish from each replicate (20 from each treatment). Wet weight (WW) was taken (to the nearest 0.01 gram) of each fish using an electronic balance. Standard length (SL, nearest 0.1 cm) was measured with the mouth closed, from the tip of the lower lip to the end of the vertebral column, and body depth (BD, nearest 0.1 cm) was taken from the base of the dorsal fin to the base of the anal fin. Fish were not fed on the day before measurements were taken.

Four ration levels were established by feeding 1%, 2%, 4% and 6% body weight $\cdot d^{-1}$ (bw $\cdot d^{-1}$). Biomass was calculated daily on achieved specific growth rates and the amount of feed adjusted to achieve the desired rations. Actual growth rates were taken at two week intervals. The ration was divided into three feedings (0900, 1500 and 2100 hours), and food was provided at these times every second day. Any uneaten food that did not exit via the drain was removed the following day. Food was provided every second day to minimize intraspecific variation in growth rates within groups of fish, and to reduce hierarchy effects common among cultured species (Irwin *et al.*, 1997). The fish were fed a diet of fry feed (C700-C1000 Biokyowa). Food selection and schedules were based on previous growth experiments at the OSC (unpublished data).

Light levels were kept at 600 lux at the water surface with a photoperiod of 18L:6D; artificial dawn and dusk. Artificial dawn and dusk was maintained by low

wattage lamps in the ceiling controlled by a timer.

This experiment lasted for 10 weeks and provided baseline data used for experiment 2.

2.2.2 EXPERIMENT 2

For experiment 2, large juveniles were used and the rations chosen were based on results from experiment 1. Prior to the experiment, fish were taken from holding tanks and transferred to experimental groups. Four different ration groups (with 2 replicates) of 100 fish each were established. Density of fish in each tank at the start was $1.45 \text{ kg}\cdot\text{m}^{-2}$, which comprised ~ 34% bottom coverage (refer to Appendix 1). Fish were placed in 0.8 m diameter black circular tanks (water depth = 35 cm) and provided with filtered sea water (mean \pm SE = $10^{\circ} \pm 0.2^{\circ}\text{C}$) at a rate of 5 L/min. (1.35 exchanges per hour).

Fish were acclimated to experimental tanks for two weeks prior to the start of the experiment. Fish were matched for weight (mean \pm SE = $7.40 \pm 0.08 \text{ g}$) and standard length (mean \pm SE = $7.32 \pm 0.03 \text{ cm}$) at the beginning of the experiment.

Growth measurements (weight and standard length) were taken at 14 day intervals on 30 fish from each replicate (60 from each treatment). Measurements were taken as described in experiment 1. Fish were not fed on the day before measurements were taken.

Four ration levels were established by feeding 1%, 1.5%, 2% and 3% body weight $\cdot\text{d}^{-1}$ ($\text{bw}\cdot\text{d}^{-1}$). Biomass was calculated daily on achieved specific growth rates and amounts

of food were adjusted to achieve the desired percentage of ration. Actual growth rates were taken at two week intervals. The ration was divided into three feedings (0900, 1500 and 2100 hours). Food was provided at these times every second day, and any uneaten food removed the following day. The fish were fed a diet of Moore -Clark's Nutra Fry 1.0 crumble, 1.5 & 2.0 mm dry feed pellet. Feed type was different from experiment 1 because import regulations made it difficult obtain BioKyowa from Japan. Additionally the high cost and poor growth results obtained in experiment 1 and its lack of availability in a larger size all contributed to this change in diet. Light levels were kept at 120 lux (lower than experiment 1 based on personal observation that fish do not need high light intensities) at the water surface and a photoperiod of 18L:6D; artificial dawn and dusk. Artificial dawn and dusk was maintained by low wattage lamps in the ceiling controlled by a timer.

Prior to the experiment, 20 whole fish were randomly sampled and frozen for initial body composition/nutrient analysis (completed in triplicate) of dietary moisture, crude protein, crude lipid, ash, carbohydrates and gross energy. At the end of the study, 20 whole fish were randomly selected from each treatment (treatments pooled) for final body nutrient analysis (completed in triplicate). Proximate analysis of diets was also sampled for dietary moisture, crude protein, crude lipid, ash, carbohydrates and gross energy (completed in triplicate) and are tabulated and compared to the manufacturers guaranteed analysis.

Nutrient analyses on chemical composition of diets and whole-body fish were done

by standard methods. The moisture content was obtained by placing pre-weighed samples in an oven set at 105 °C until the dry weight was constant (24 hours.). Samples were cooled in a desiccator to eliminate moisture. The protein content of dried samples was determined using the Kjeldahl method (Tecator Digestion System 20, 1015 digester, Sweden; Tecator Kjelttec System 1028 Distilling Unit, Sweden). Total nitrogen was converted to protein by multiplying by 6.25 on the assumption that the protein in the feed is approximately 16% nitrogen. The crude lipid content was carried out using a hexane-based Soxhlet lipid extraction apparatus (Tecator Soxtec System HT 1043 Extraction Unit, Sweden). The ash content was measured by placing pre-weighed crucibles with either the dried diet or body samples in a muffle furnace (Thermolyne, Sybron Corporation, Dubuque, Iowa, USA) set at @450°C for 24 hrs., cooling in a desiccator and re-weighing the crucible and sample. The carbohydrate content was determined by subtracting the sum of the other nutrients from 100 (Goddard, 1992). The gross energy was calculated by multiplying the percent protein by 5.6 kcal/gram, percentage lipid by 9.5 kcal/gram and percentage carbohydrate by 4.1 kcal/gram (Goddard, 1992). These values are the gross energy per calorie for each of protein, lipid and carbohydrate. The sum of these values equals the gross dietary energy per 100 grams. Diets were analysed using the same methods.

2.2.3 DATA ANALYSIS

Data on mean weight (g), standard length (mm), body depth (mm), specific growth

rate (SGR), gross food conversion ratio (GFCR), coefficient of variance (CV %), Fulton's condition factor (K), stocking density or percent bottom coverage (kg·m⁻² or %), body composition, food composition and survival were collected (see Appendix 1).

Data sets for both experiments 1 & 2 were analysed using SAS/STAT (SAS Institute, 1988). A nested analysis of variance (ANOVA) (Zar, 1982) was used to test for tank effects. A General Linear Model (GLM) determined if age or treatment influenced the growth parameters of juveniles under different treatments (rations) and for each growth measurement. Homogeneity of slopes was tested using interaction terms and if no significant interactions were found, an analysis of covariance was performed.

Residual plots were examined for equality of variance and normality of the data. For data where equality of variance was not satisfied, the data were log transformed. Analysis of covariance (ANCOVA) was followed by Tukey's multiple comparison test ($\alpha=0.05$). All statistical tests were deemed acceptable, as the residuals were found to be independent of the model, and normal in distribution (Sokal & Rohlf, 1995).

2.3 RESULTS

Mortalities were low (1/400= 0.25% in experiment 1 and 14/800 = 1.75% in experiment 2) and were not analysed (Tables 2.1 and 2.2). Coefficients of variation (CV%) between rations were also minimal between treatments in both experiments (Figures 2.1 a, b, c and 2.5 a, b) which suggests food was not limited.

2.3.1 EXPERIMENT 1

The coefficient of variance (% CV) for weight and length remained relatively unchanged over the 10 week experimental period (Figures 2.1 a, b, c).

A nested ANOVA showed no significant differences (ie. no tank effect) between the replicates of treatments for weight ($df=4$; $F=0.93$; $p=0.444$), standard length ($df=4$; $F=0.72$; $p=0.579$), or body depth ($df=4$; $F=0.25$; $p=0.907$) and subsequently data were pooled. Significant differences ($\alpha=0.05$) were found among rations for weight ($df=3$; $F=4.04$; $p=0.008$), standard length ($df=3$; $F=3.30$; $p=0.020$) and body depth ($df=3$; $F=4.21$; $p=0.006$) when data was analyzed using ANCOVA, with week as the covariable. However, no significant differences among treatments ($p > 0.05$) were found when data were analyzed bi-weekly using ANOVA.

Results indicated that juveniles fed a ration of 1% $bw \cdot d^{-1}$ had significantly lower growth (mean final weight, length and body depth) than those fed 2%, 4% and 6% $bw \cdot d^{-1}$ (Table 2.1 ; Figures 2.2 a, b, c). A ration of 1% was close to being significantly different ($df=3$; $F=6.50$; $p=0.051$) from all other rations in terms of a lower specific growth rate (SGR's- Figure 2.3). Significant differences ($df=3$; $F=160.9$; $p < 0.05$) in gross food conversion ratios (GFCR's) were found between fish fed rations of 1% and 2% and those fed 4% and 6% rations (Table 2.1 and Figure 2.4).

Table 2.1 Mean initial weight (g \pm SE), mean final weight (g \pm SE), initial bottom coverage (%), final bottom coverage(%), initial condition (K) factor, final condition (K) factor, specific growth rate (% per day SGR), gross food conversion ratios (GFCR) and survival (%) for 0' yellowtail flounder fed four different rations of 1%, 2%, 4%, 6% body weight \cdot d⁻¹ (bw \cdot d⁻¹), (N= 20 per treatment).

Ration Group (bw\cdotd⁻¹)	Mean Initial Weight (g)	Mean Final Weight (g)	Initial Bottom Coverage (%)	Final Bottom Coverage (%)	Initial K-factor	Final K-factor	Specific Growth Rate (% per day)	Gross Food Conversion Ratio's (GFCR)	Survival (%)
1%	1.03 \pm .09	1.80* \pm .15	46	66	1.67	1.70	0.80	1.19*	100
2%	1.01 \pm .07	2.33 \pm .17	44	78	1.70	1.71	1.20	1.58*	100
4%	0.94 \pm .07	2.14 \pm .19	43	73	1.65	1.72	1.19	3.34	100
6%	1.01 \pm .09	2.39 \pm .21	42	78	1.69	1.76	1.25	4.45	99

*****, Indicates a significant difference between the treatments (P<0.05). (Tukey's multiple comparison test).

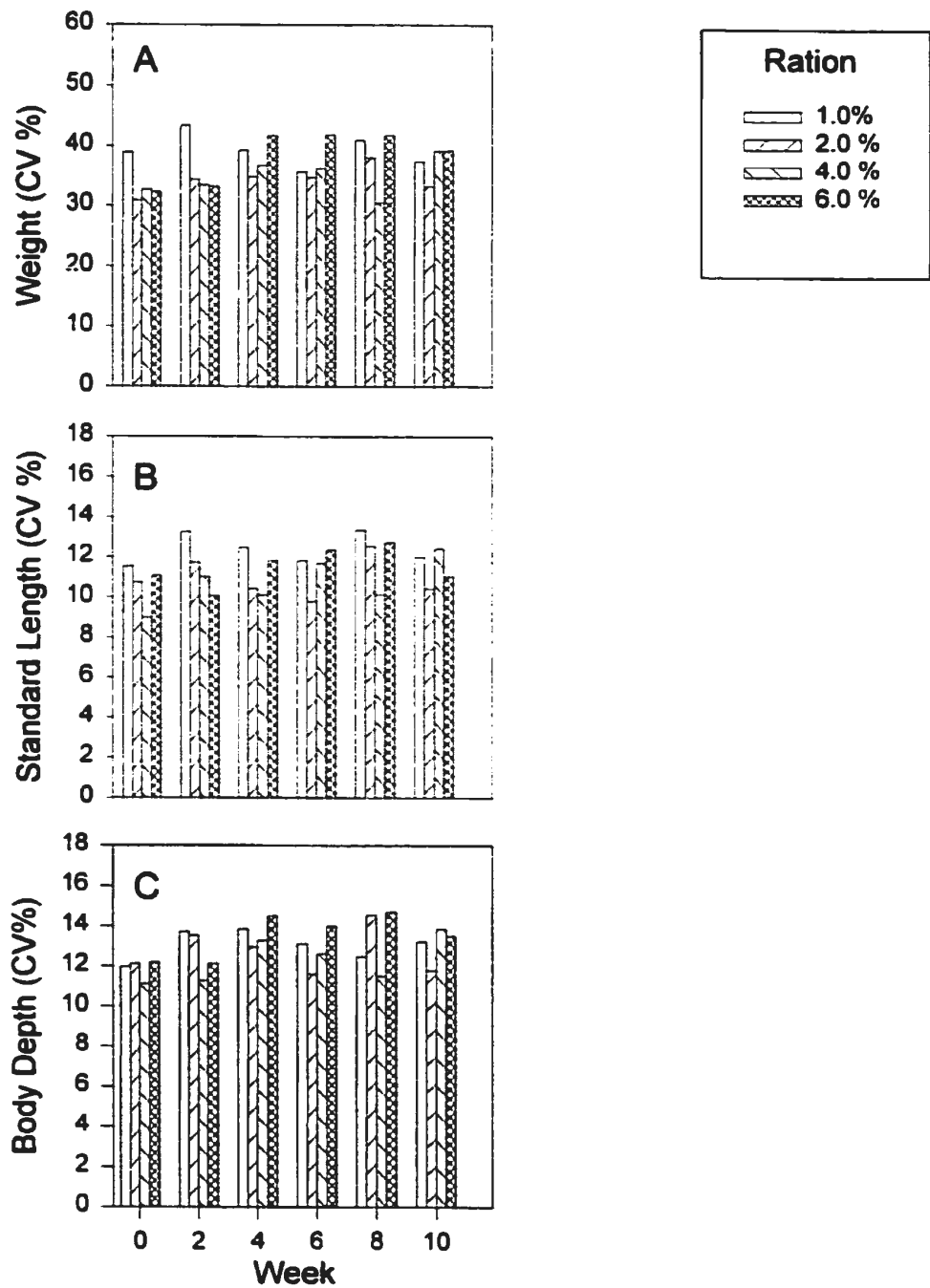


Figure 2.1 Mean coefficient of variation (%) for A) weight (g), B) standard length (cm) and C) body depth (cm) of 0+ yellowtail flounder fed four different rations of 1%, 2%, 4% and 6% body weight * day⁻¹. N=20 per treatment.

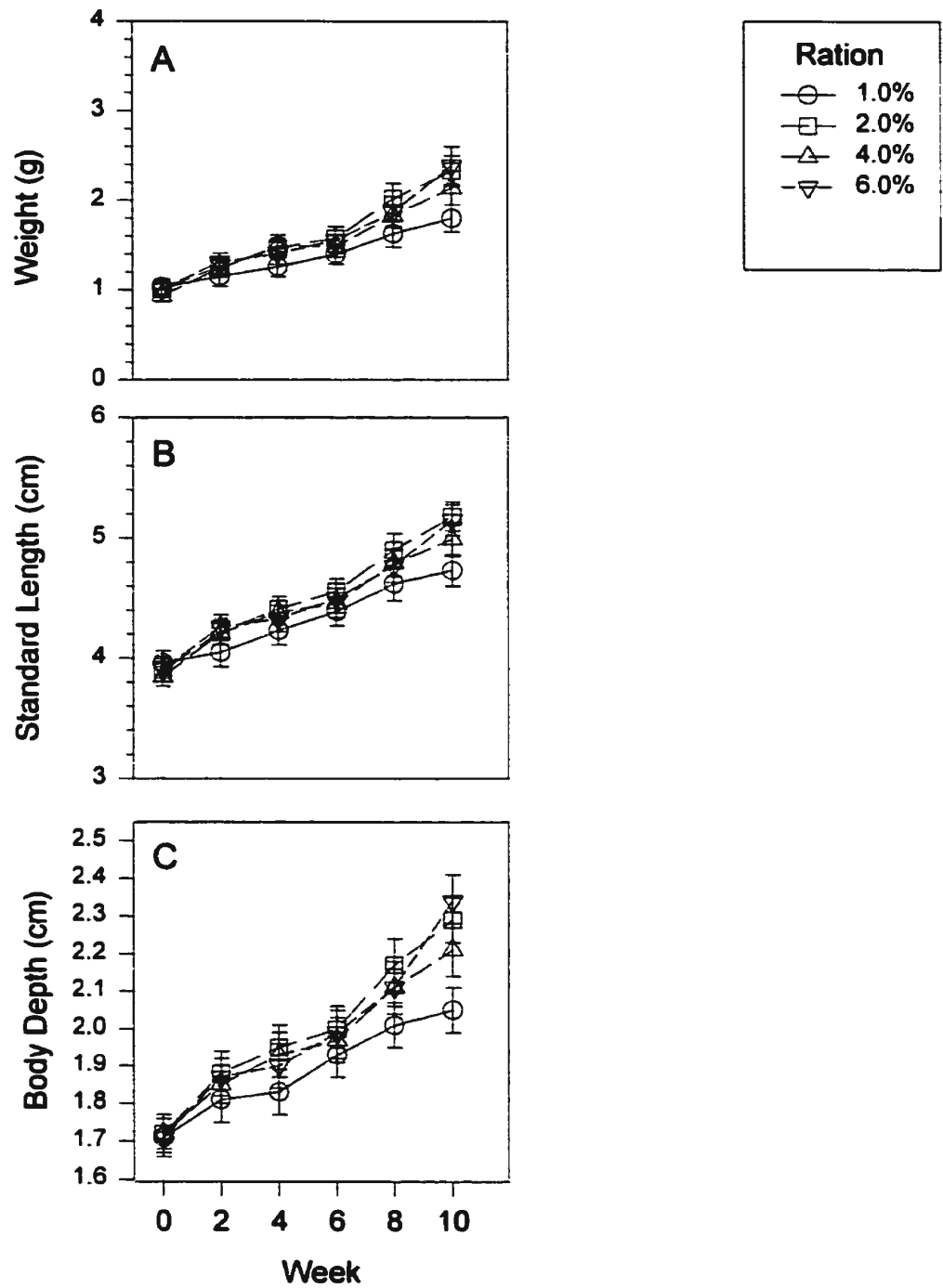


Figure 2.2 A) Mean weight (g), B) mean standard length (cm) and C) mean body depth (cm) of 0+ yellowtail flounder fed four different rations of 1%, 2%, 4% and 6% body weight \times day⁻¹. Vertical bars represent standard error. N= 20 per treatment.

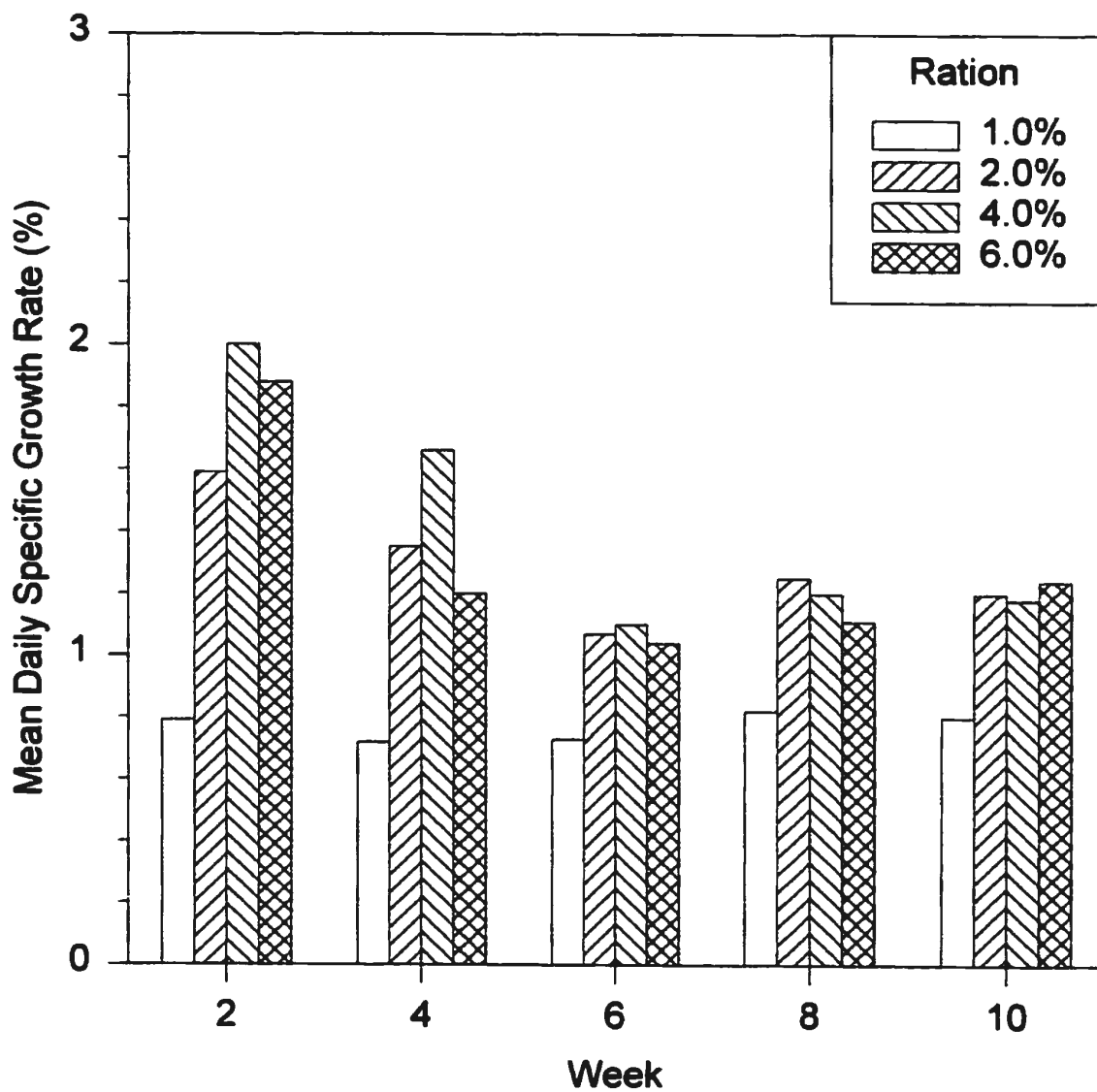


Figure 2.3 Mean daily specific growth rate for 0+ yellowtail flounder fed rations of 1.0%, 2.0%, 4.0% and 6.0% bw * day⁻¹.

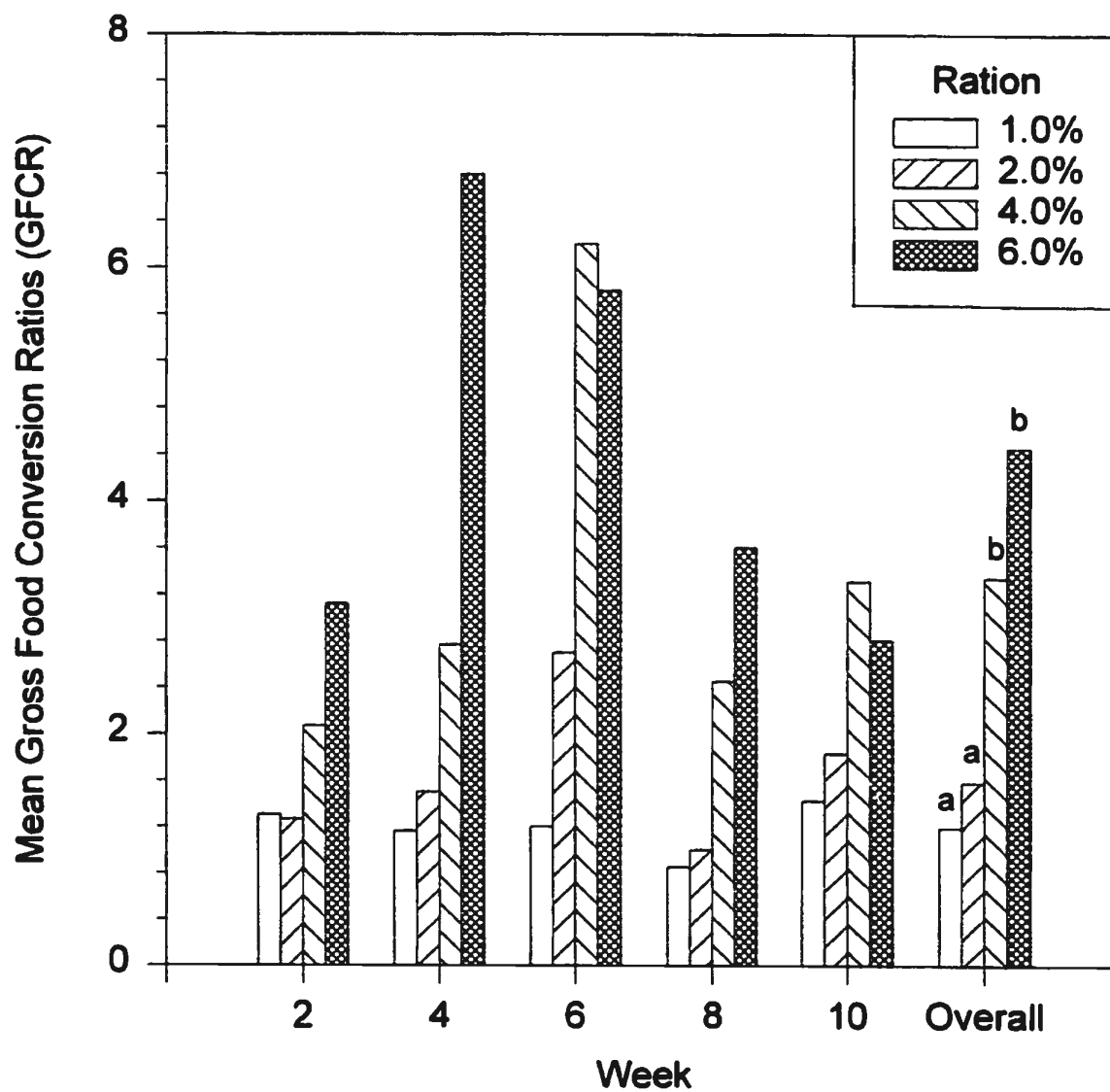


Figure 2.4 Mean gross food conversion ratio of 0+ yellowtail flounder fed rations of 1.0%, 2.0%, 4.0% and 6.0% body weight * day⁻¹. Different letters indicate significant differences at $\alpha=0.05$.

2.3.2 EXPERIMENT 2

The coefficient of variance (CV %) for weight and length increased slightly over the first 6 weeks, increased sharply in week 8 and stabilized for the remaining 6 week experimental period (Figures 2.5 a, b).

A nested ANOVA showed no significant differences (ie. no tank effect) between the replicates of treatments for either weight ($df=4$; $F=1.04$; $p=0.384$) or standard length ($df=4$; $F=0.64$; $p=0.634$) and subsequently data were pooled. Significant differences ($\alpha=0.05$) were found among rations for weight ($df=3$; $F=6.81$; $p<0.05$) and standard length ($df=3$; $F=5.44$; $p=0.001$) when data were analyzed using ANCOVA, with week as the covariable.

There were no significant differences in the specific growth rates of any group ($df=3$; $F=4.12$; $p=0.103$). Fish fed a ration of 3% $bw \cdot d^{-1}$ had higher (even though not significant) growth rates than those fed 1%, 1.5% and 2% $bw \cdot d^{-1}$ (Table 2.2 , Figures 2.6 a, b). The specific growth rates (SGR's) were higher for fish fed 3% as compared to 1%, 1.5% and 2% rations (Table 2.2 and Figure 2.7). Gross food conversion ratios (GFCR's) were significantly different ($df=3$; $F=1004.15$; $p<0.05$) for all 4 rations. It was lower for 1% than 1.5%, 2% and 3% rations (Table 2.2 and Figure 2.8).

Analysis of whole body nutrient composition of yellowtail showed no significant differences ($p>0.05$) among treatments for moisture ($df=4$; $F=2.63$; $p=0.098$), lipid ($df=4$; $F=2.20$; $p=0.142$), carbohydrates ($df=4$; $F=2.98$; $p=0.074$) or gross energy ($df=4$; $F=3.10$; $p=0.067$). However, significant differences were found for protein ($df=4$; $F=3.69$;

Table 2.2 Mean initial weight (g \pm SE), mean final weight (g \pm SE), initial bottom coverage (%), final bottom coverage(%), initial condition (K) factor, final condition (K) factor, specific growth rate (% per day SGR), gross food conversion ratios (GFCR) and survival (%) for 0+ yellowtail flounder fed four different rations of 1%, 1.5%, 2%, 3% body weight $\cdot d^{-1}$ (bw $\cdot d^{-1}$), (N= 60 per treatment).

Ration Group	Mean Initial Weight (g)	Mean Final Weight (g)	Initial Bottom Coverage (%)	Final Bottom Coverage (%)	Initial K-factor	Final K-factor	Specific Growth Rate (% per day)	Gross Food Conversion Ratio (GFCR)	Survival (%)
1%	7.39 \pm .17	21.26 \pm .79	34	66	1.87	1.88	0.95	1.02*	99
1.5%	7.37 \pm .15	21.20 \pm .78	34	68	1.86	1.84	0.95	1.50*	98
2%	7.39 \pm .16	21.71 \pm .76	33	68	1.90	1.89	0.97	1.95*	98
3%	7.46 \pm .15	23.15* \pm .89	34	70	1.88	1.93	1.02	2.86*	99

*, Indicates a significant difference between the treatments ($P < 0.05$). (Tukey's multiple comparison test).

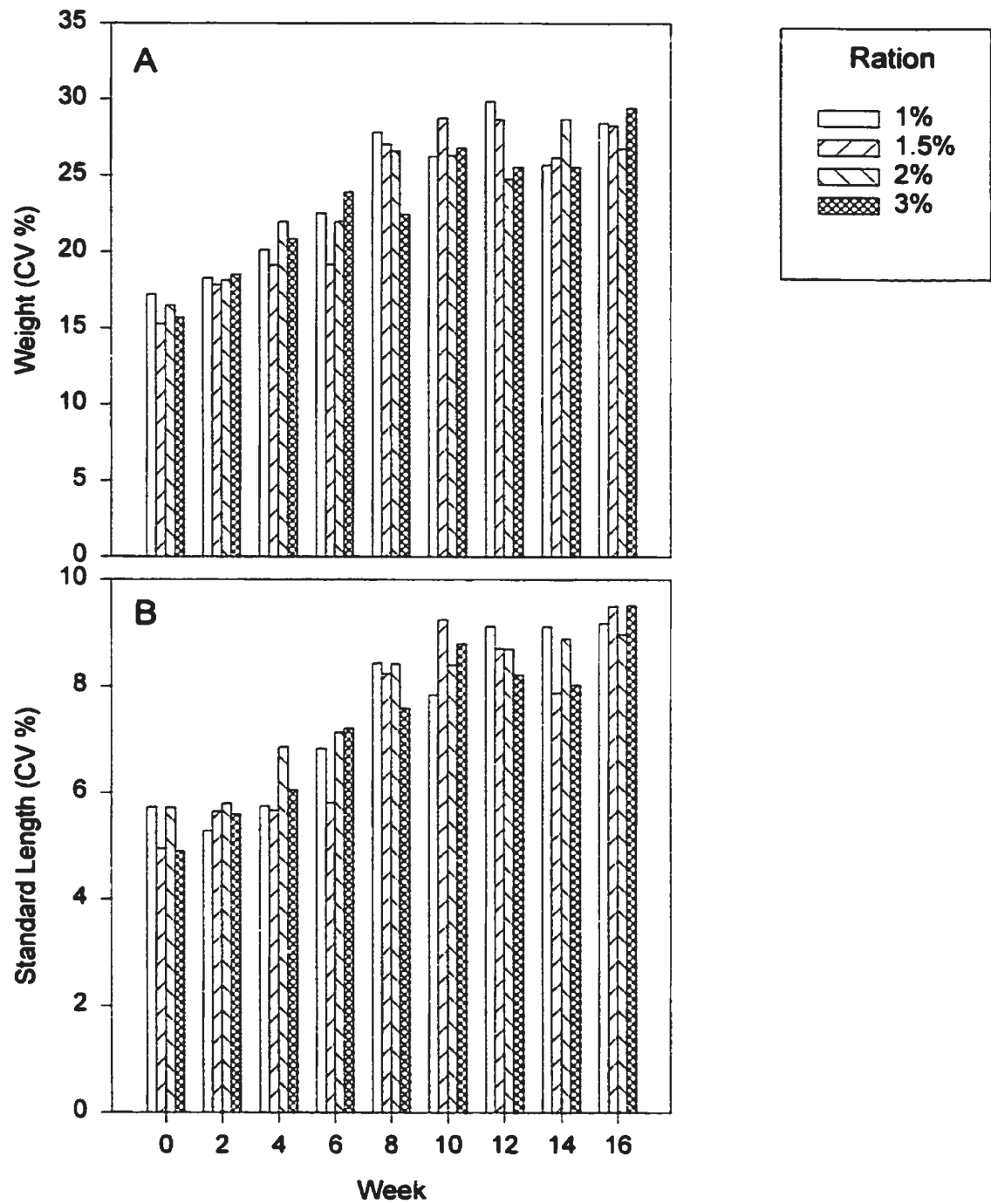


Figure 2.5 Mean coefficient of variation (%) for A) weight (g) and B) standard length (cm) of 0+ yellowtail flounder fed four different rations of 1%, 1.5%, 2% and 3% body weight \times day⁻¹. N= 60 per treatment.

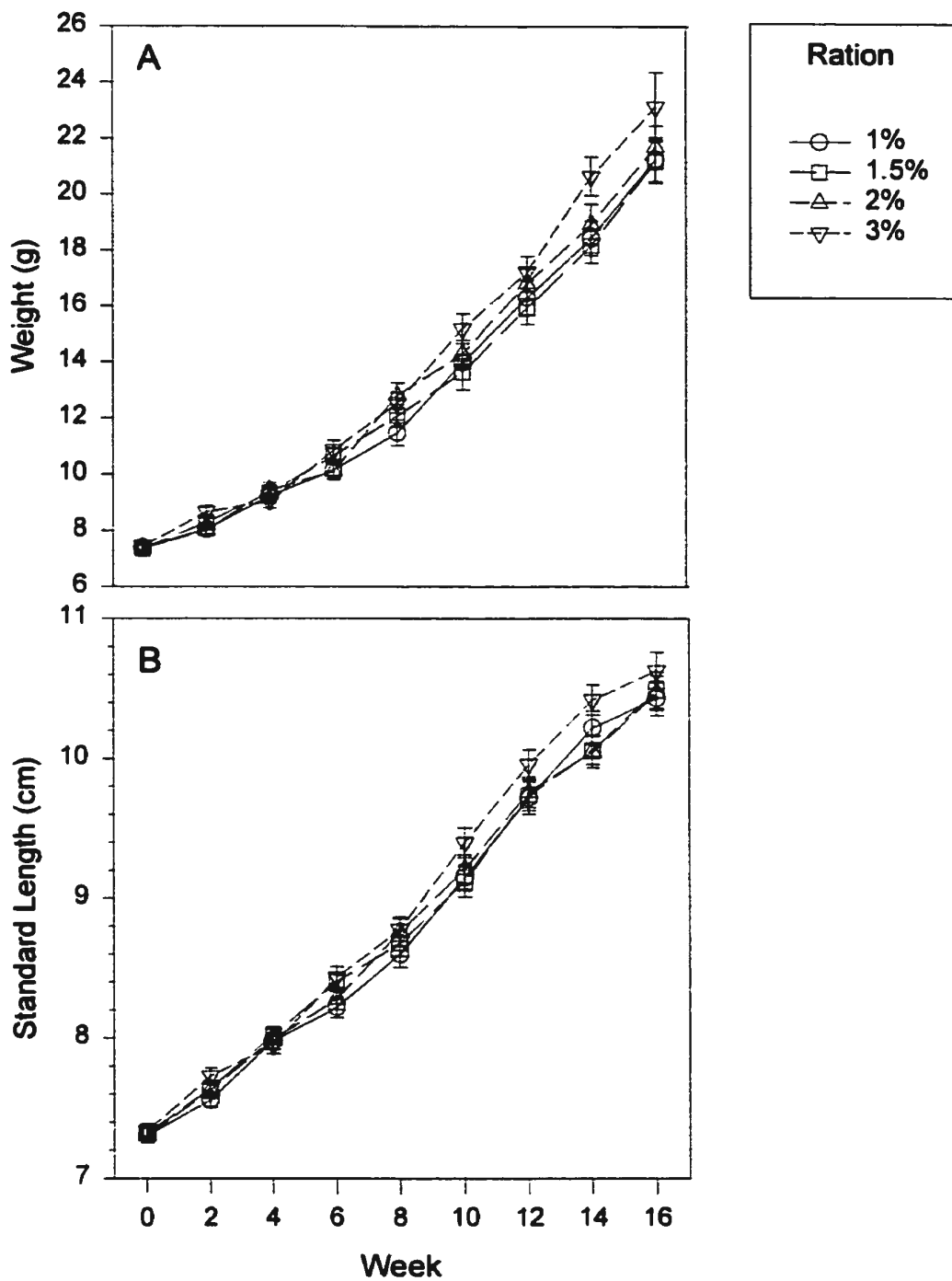


Figure 2.6 A) Mean weight (g) and B) mean standard length (cm) of 0 + yellowtail flounder fed four different rations of 1%, 1.5%, 2% and 3% body weight \times day⁻¹. Vertical bars represent standard error. N= 60 per treatment.

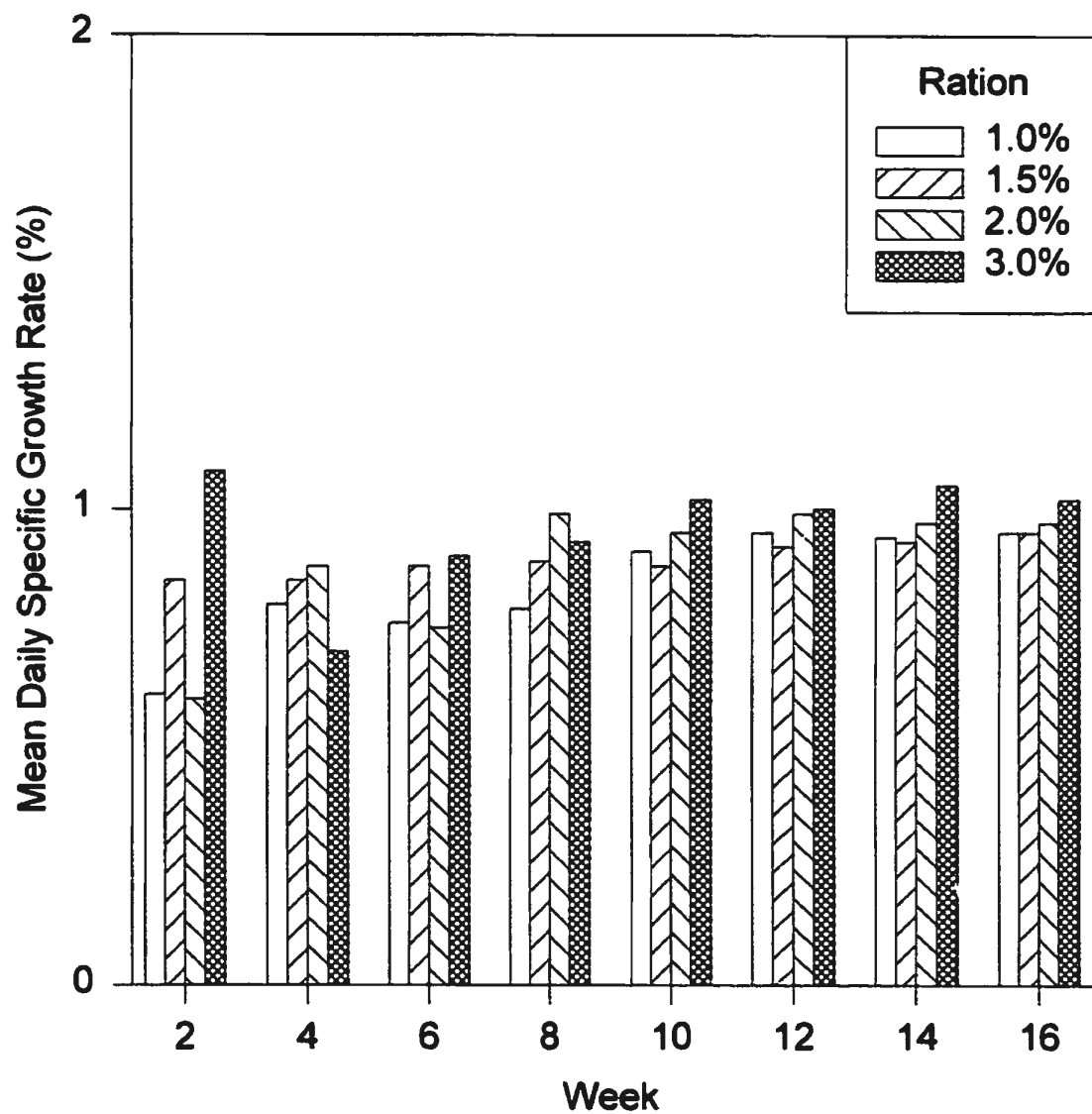


Figure 2.7 Mean daily specific growth rate for 0+ yellowtail flounder fed rations of 1% , 1.5%, 2.0% and 3.0% body weight * day⁻¹.

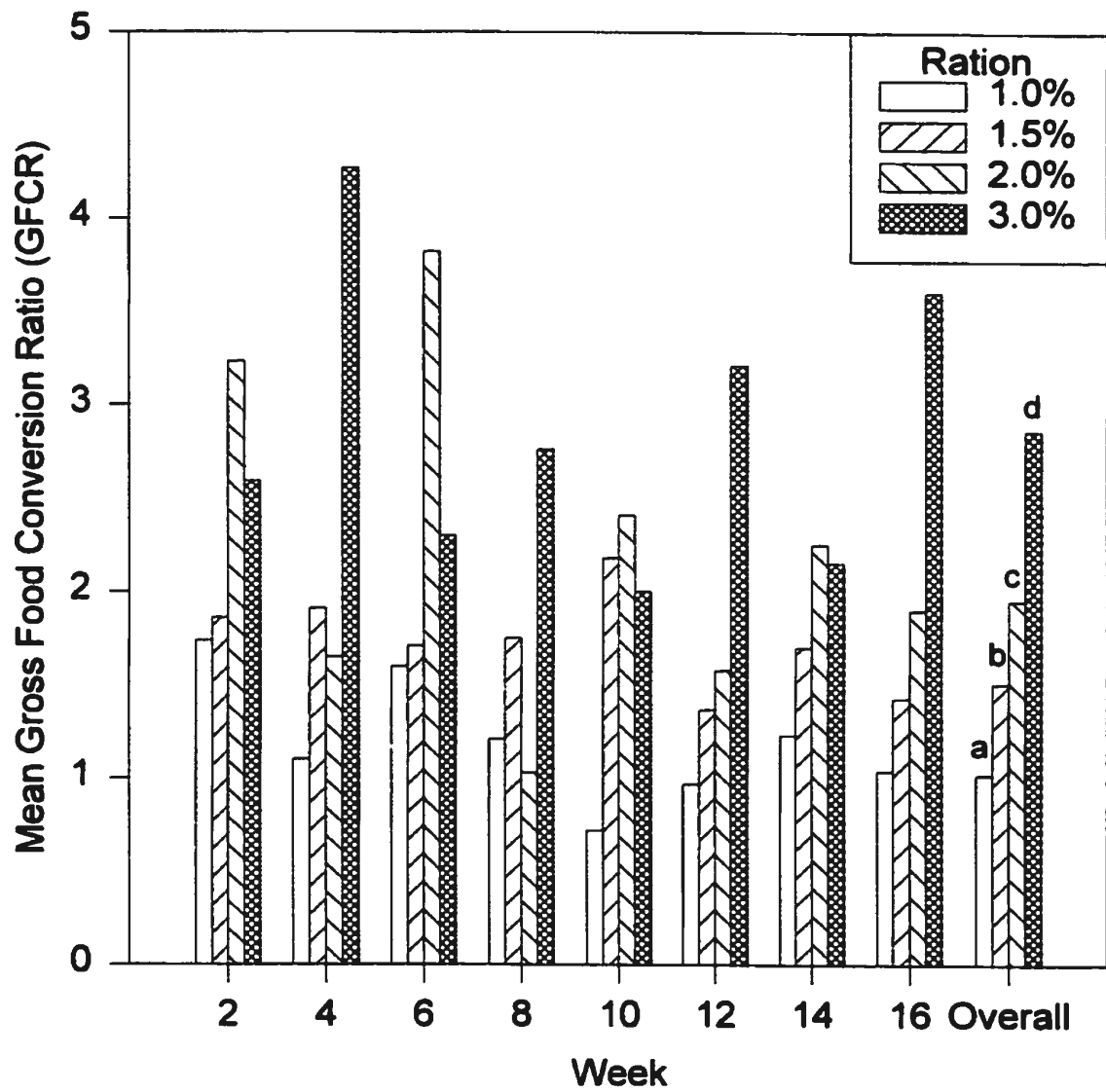


Figure 2.8 Mean gross food conversion ratios of 0+ yellowtail flounder fed rations of 1%, 2%, 4%, and 6% body weight \times day⁻¹. Different letters indicate significant differences at $\alpha=0.05$.

p=0.043) and ash content (df=4; F=5.11; p=0.017) (Table 2.3).

Table 2.3 Mean values of whole-body nutrient composition for % body moisture, % body protein, % body lipid, % body ash, % body carbohydrates and gross dietary energy per 100 grams (kcal/g) for 0⁺ juvenile yellowtail flounder fed four different rations of 1%, 1.5%, 2%, 3% body weight·d⁻¹ (bw·d⁻¹), (N= 20 per treatment). Values are mean ± standard error (SE); n=3(complicated in triplicate).

Sample	Moisture %	Protein %	Lipid %	Ash %	Carbohydrates %	Gross Energy kcal/g
Initial	75.12 ±0.08	48.39 ±3.96*	25.96 ±0.95	13.50 ±0.28*	3.02 ±0.73	5.20 ±0.10
1.0%	75.04 ±0.27	57.94 ±1.15	23.79 ±0.99	12.35 ±0.18	1.60 ±0.24	5.43 ±0.07
1.5%	74.61 ±0.16	55.61 ±1.78	25.83 ±0.25	12.11 ±0.31	1.65 ±0.44	5.52 ±0.06
2.0%	76.07 ±0.10	61.71 ±0.51*	23.75 ±0.13	12.57 ±0.59	0.47 ±0.03	5.61 ±0.03
3.0%	74.78 ±0.54	55.54 ±1.21	26.43 ±0.73	10.78 ±0.06*	1.85 ±0.37	5.59 ±0.09

*, Indicates a significant difference between the treatments (P<0.05). (Tukey's multiple comparison test).

Nutrient analysis of the diets (Moore-Clark Nutra Plus Crumble and Nutra Fry dry feed) used in this experiment shows that the manufacturer's guaranteed analysis is very close to what is being delivered and provided on the label (Tables 2.4 a, b).

Table 2.4 a Nutrient analysis of diet (Salmonid starter feed, Moore-Clark Nutra Plus Crumble and Nutra Fry Pellet) fed to 0⁺ juvenile yellowtail flounder in ration experiment 2.¹ Values are mean ± standard error (SE); n=3 (complicated in triplicate).

Sample	Moisture %	Protein %	Lipid %	Ash %	Carbohydrate %	Gross Energy kcal/g
#1crum.	5.24 ± 0.00	54.83 ± 0.74	16.94 ± 0.34	10.45 ± 0.02	12.54 ± 0.48	5.09 ± 0.01
1.5 mm	6.33 ± 0.02	52.82 ± 0.26	25.07 ± 0.10	7.88 ± 0.03	7.88 ± 0.30	5.56 ± 0.01
2.0 mm	7.76 ± 0.02	51.02 ± 0.25	21.50 ± 0.55	6.96 ± 0.01	12.77 ± 0.78	5.32 ± 0.03

Table 2.4 b Manufacturer's (Moore-Clark) guaranteed analysis of fish fry feed ^{1, 2} fed to 0+ juvenile yellowtail flounder in ration experiment 2 (Section 2.2.2).

Manufacture's Guaranteed Analysis	Protein Minimum (%)	Lipid Minimum (%)	Ash Maximum (%)
#1 crumble	52.00	20.00	10.00
1.5 mm	48.00	24.00	7.00
2.0 mm	48.00	24.00	7.00

¹ Salmonid starter feed, Moore-Clark Nutra Plus Crumble and Nutra Fry.

² Ingredients: Fish meal, fish oil, whole wheat, krill meal, blood meal, betaine, lecithin, vitamin premix, mineral premix.

2.4 DISCUSSION

The economics of aquaculture is an area of increasing interest to fish farmers. In contrast to agriculture, aquaculture has less history in regard to its economics. This is mainly due to the relative youth of the aquaculture industry and the fact that development up to now has been mostly technology based (Neiland, 1994). There is also a widely held belief among scientists that they should "get the science right first" before involving other disciplines such as economics (Neiland, 1994). Science is very important, but baseline data from other disciplines (economics) can be equally as important.

Experiment 1 demonstrated that 0+ juvenile yellowtail flounder fed a 1% d⁻¹ ration were smaller (mean final and specific growth rate) than those fed a 2%, 4% or 6% bw·d⁻¹ ration. Fish fed rations of 2%, 4% or 6% bw · d⁻¹ showed greater specific growth rates, but rations of 4%-6% bw·d⁻¹ were poor in terms of gross food conversion ratios, thus

resulting in food wastage. A 2% bw·d⁻¹ ration is more appropriate in terms of growth and efficient food conversion ratios for small 0+ yellowtail flounder juveniles held under the above conditions.

Experiment 2 indicated that large juvenile fish fed a 3% bw·d⁻¹ ration had higher growth (mean final and specific growth rate) than fish fed a 1%, 1.5% or 2% bw·d⁻¹ ration. Fish fed a ration of 3% bw·d⁻¹ were better in terms of specific growth rates, but not as good in terms of gross food conversion ratios which resulted in food wastage. Therefore, a 1.0 %-1.5 % bw·d⁻¹ ration appears more economical in terms of growth and efficient food conversion ratio for larger 0+ yellowtail flounder juveniles held under the above conditions.

Both experiments indicated that maximum conversion efficiency occurs at ration levels below those at which maximum growth occurs as referenced by Goddard, 1996. It is evident that there is a range of possible feeding levels, the choice of which depends on whether maximum growth, optimal food conversion, or a balance between the two is sought.

As indicated in both experiments, when using high ration levels of 3%, 4%-6 % bw·d⁻¹ ration, gross food conversion ratio (GFCR) increased to 2.86 - 4.45 indicating a poor food conversion ratio. At lower ration levels of 1-2 % bw·d⁻¹ rations, gross food conversion ratio (GFCR) decreased to 1.02-1.95, indicating a better food conversion ratio. As described above, it is advisable to achieve a feeding level which balances both growth and food conversion.

Ration can change with size, age and temperature. Daily specific growth rates and gross food conversion ratios may be valuable indicators of food ration. My results are similar to those feeding studies which have been conducted with other flatfish and non-flatfish species. Hatanaka *et al.*, (1956) found for two pleuronectids that a ration of 1.12% d⁻¹ was adequate for 42-279 g *Limanda yokohamae*, and 0.95% was adequate for 87-234 g *Kareius bicoloratus* at temperatures of 13-15°C. Frame (1972) reported a maintenance ration of 1.5% d⁻¹ for 30-100 g winter flounder, *Pseudopleuronectes americanus* under 12-20°C. Tyler and Dunn (1976) showed that food intake with adult winter flounder was 2% d⁻¹ under *ad libitum* feeding conditions. It has also been shown with groups of large Japanese plaice (*Kareius bicoloratus*) that they consume mean rations of 1.21-6.43 % d⁻¹ under *ad libitum* feeding conditions, the rations varying inversely with body size and directly with temperature (Hatanaka *et al.*, 1956). Quinton and Blake (1990) showed with 36.24 g (mean weight) rainbow trout *Oncorhynchus mykiss* (Walbaum), that all groups of fish were overfed at rations of 3%, 5% and 7% bw·d⁻¹. Increased rations only decreased conversion efficiency, indicating overfeeding as found with the higher rations I used in my experiments.

In this study (with some low ration allocations) feed was provided every second day with the intention to minimize variation in growth rates within groups of fish and to reduce hierarchy effects common under laboratory conditions (Magnusson, 1962; Eaton & Farley, 1974; Jobling, 1982; Abbott *et al.*, 1985; Ruzzante, 1994; Irwin *et al.*, 1997). If fish are fed meals of reduced ration on a daily basis, then larger or more aggressive fish

may feed at the expense of smaller fish (Goddard, 1996). It is possible for larger fish to feed at maximum rates, while others in the same group are feeding on restricted rations. Differences in body size could have a significant impact upon an individual's ability to compete socially and may be reflected in growth being suppressed. In light of these works, feeding regimes were selected to give all fish equal opportunities to eat when food was provided and thus reduce or abolish size hierarchy effects.

Coefficients of variation between treatments was minimal (weight and length) in both experiments, which seems to indicate that fish were not deprived of feed. Growth dispensation has been reported for flatfish held in groups (Purdom, 1974; Ehrlich *et al.*, 1976). Carter *et al.* (1996) suggested that individual differences in food consumption, mediated through exploitation competition, contribute to growth dispensation in greenback flounder *Rhombosolea tapirina*. Pittman (1998) stated that many flatfish have lower ingestion rates and food requirements after metamorphosis because of higher food conversion efficiency.

Nutrient analysis of diets which provides an indication of levels of moisture, protein, fat, ash, carbohydrate and energy, may aid nutritionists in modifying the composition of the carcass in hatchery situations to meet consumer demands (Shearer, 1994). Analysis of whole body nutrient composition of yellowtail showed no significant differences ($p>0.05$) between treatments with moisture, lipid, carbohydrates or gross energy. Significant differences were found between initial and final fish sampled using a 2% bw·d⁻¹ ration with protein, and 3% bw·d⁻¹ ration with ash.

Nutrient analysis of diets (Moore-Clark Nutra Plus Crumble and Nutra Fry dry feed) clearly showed that the stated analysis of fish food used in this experiment was close to that being delivered as the final product, and to that written on the food label attached to the bag. This is an important consideration since producers try to feed their fish the best diets available, which in turn may improve growth rates and survival of fish.

Attention must also be focussed on diets, because when high-energy, lipid rich food pellets are used in aquaculture, the fish tend to build up excess lipids, resulting in fish with high fat content. However, this was not seen in my experiments. The increased use of high fat diets in aquaculture has increased the need for future studies to focus on the effect of feed formulation (protein energy, total energy) on the deposition of growth energy of hatchery-reared juveniles (Grant *et al.* 1998).

Research directed towards fish nutrition is important and should continue in order to optimize the effects of dietary protein, carbohydrate and fat levels when using cold water marine finfish feeds. Protein supplements and fish oils are expensive components of commercial fish diets. Hjertnes *et al.* (1991) showed that growth is reduced when protein levels are replaced by carbohydrates and/or fat for juvenile Atlantic halibut. Protein is more expensive to incorporate into diets than lipids. Unfortunately, feed manufacturing companies are producing marine diets which are lower in protein (minimum 48%) and higher in lipids (minimum 20%). Hjertnes and Opstvedt (1989) and Hjertnes *et al.* (1991) showed that juvenile halibut have a food requirement of at least 58% dietary protein. Cowey *et al.* (1972) found optimal dietary protein to be 50% for plaice, while Lie *et al.*

(1988) found maximum growth rates in cod with 60% dietary protein.

My study indicates that feed rations are important in determining the growth performance of fish. Rations should accommodate the short term fluctuations which occur in appetite, and should be adjusted to meet the changing feed demands as fish grow or water temperatures and other environmental factors change (Goddard, 1996). Feeding the yellowtail flounder juveniles restricted rations in these experiments demonstrated a greater control of gross food conversion ratios. An understanding of feed rations and feed conversions is fundamental in developing cost effective feeding programs which are aimed at maximizing food conversion efficiencies and growth. By optimizing feeding strategies, economic benefits can be realized.

Gross food conversion ratios indicate that economically it would be more feasible to use a ration of 2 % $\text{bw} \cdot \text{d}^{-1}$ for small juveniles and 1-1.5% $\text{bw} \cdot \text{d}^{-1}$ for larger juveniles. It is also important to feed diets which contain optimal levels of dietary protein, carbohydrate and fat levels when using cold water marine finfish feeds.

CHAPTER 3

3.1 INTRODUCTION

Information concerning the effects of environmental factors, such as photoperiod, on the growth of juvenile flatfish is limited. Jones (1973) found that growth of juvenile turbot showed a distinct seasonal pattern: higher in summer than in winter. It was later suggested that this growth pattern was partly due to seasonal changes in day length (Imslund *et al.*, 1995). Photoperiod acts as a zeitgeber, entraining endogenous rhythms in fishes (Duston & Saunders, 1990). It has been shown to affect growth, locomotor activity, smolting, and sexual maturation in some species of fish (Villarreal *et al.*, 1988). This can be a result of the rate of change of day length (differential effect) or the absolute day length (proportional effect) (Eriksson & Lundqvist, 1980).

Extended photoperiod has resulted in increased growth of juvenile Atlantic salmon, *Salmo salar*, in freshwater (Saunders *et al.*, 1989; Stefansson *et al.*, 1989; Berg *et al.*, 1992) and post smolts in seawater (Saunders & Harmon, 1988; Kråkenes *et al.*, 1991). Corroborative evidence for the growth-promoting effect of extended photoperiod has been demonstrated in studies with green sunfish, *Lepomis cyanellus* (Gross *et al.*, 1965), plaice, *Pleuronectes platessa*, and sole, *Solea solea* (Fonds, 1979), juvenile splitnose rockfish, *Sebastes diploproa* (Boehlert, 1981), Atlantic cod, *Gadus morhua*, (Folkvord & Otterå, 1993) and winter flounder, *Pseudopleuronectes americanus* (Casey & Litvak, unpublished data).

Fuchs (1978) who worked with juvenile sole, *Solea solea*, found no significant differences in growth and survival between 12, 18 and 24 h light. Kiyono and Hirano

(1981) reported that juvenile growth did not significantly differ with black porgy, *Mylio macrocephalus* under 13, 18 and 24h light. Surprisingly, there is little literature on the biology of larvae and juveniles as it relates to survival and growth in hatcheries (Barlow *et al.*, 1995). No research on juvenile yellowtail flounder with respect to photoperiod is currently available. The purpose of this study was to determine a photoperiod which promotes the best growth and survival of juvenile yellowtail flounder.

3.2 MATERIALS AND METHODS

Yellowtail flounder oocytes and sperm were obtained from captive broodstock, held at Memorial University's Ocean Sciences Centre, in Logy Bay, Newfoundland. Fertilized embryos were incubated in 200 Litre conical incubators and supplied with air and seawater. First feeding larvae were fed cultured rotifers and Artemia. Metamorphosing larvae were transferred to 1 metre circular tanks (depth 40 cm), and weaned onto artificial food (Fry Feed Kyowa). Larvae and young juveniles were maintained under 24 and 12 hours light per day respectively.

Prior to the experiment, fish were taken from large holding tanks (12L:12D) and transferred to experimental groups. Four groups (with 2 replicates) of 40 fish were established and the density of fish in each tank at the start was 0.47 kg·m⁻², which comprised 10% bottom coverage of fish (refer to Appendix 1). Fish were placed in square tanks (90 x 90cm with 30 cm water depth), provided with filtered salt water (mean ± SE = 7° ± 0.3°C) flowing at a rate of 6 L/min. for a tank exchange rate of 1.48 times per

hour. Fish were matched for weight (mean \pm SE = 9.25 ± 0.22 g), standard length (mean \pm SE = 7.99 ± 0.07 cm) and body depth (mean \pm SE = 3.58 ± 0.03 cm) at the beginning of the experiment.

Lighting was supplied by 60 Watt (incandescent) bulbs placed 30 cm from the water in the middle of the tank, which distributed light evenly to all tank areas. Light intensity was 150 lux at the water's surface. Lights were turned on and off by automatic timers and came on at 0900 hr. Treatment 1 received 24 hours of light per day (24Light:0Dark), treatment 2 - 18 hours (18L:6D), treatment 3 - 12 hours (12L:12D) and treatment 4 - ambient photoperiod (for this treatment, the photoperiod was adjusted every week (to the nearest 1/4 hour) to our latitude 47°N beginning September 30).

Fish were fed a formulated pellet (Fry Feed Kyowa-C3000) over a 30–40-min. period (apparent satiation) twice daily during the light phase (of the shortest photoperiod) at a ration of 2% body weight·d⁻¹ (bw·d⁻¹). A 2% bw·d⁻¹ ration proved to be significantly above maintenance (high ration excess), which was indicated by some food remaining in the tanks after feedings. Previous experiments (Dawes, 1930 a, b, c; Bromley, 1974) indicated that sole were satiated and growth was promoted at a 2% bw·d⁻¹ ration. (Also refer to Tables 2.1 and 2.2 in Chapter 2). Biomass was calculated daily on achieved specific growth rates to ensure a 2% bw·d⁻¹ ration.

Growth measurements (weight, standard length and body depth) were taken at 21 day intervals on 20 fish from each group (40 from each treatment). The wet weight (WW) was taken (to the nearest 0.01 gram) of each sampled fish using an electronic balance.

Standard length (SL, nearest 0.1 cm) was measured with the mouth closed, from the tip of the lower lip, along the lateral line, to the end of the vertebral column, and body depth (BD, nearest 0.1 cm) was taken from the base of the dorsal fin to the base of the anal fin. Fish were not fed on the day before measurements were taken.

3.2.1 DATA ANALYSIS

Data were analysed using SAS/STAT (SAS Institute, 1988). A nested analysis of variance (ANOVA) (Zar, 1982) was used to test for tank effects. A General Linear Model (GLM) determined if age or treatment influenced the growth parameters of juveniles under different treatments (photoperiods) and for each growth measurement. Homogeneity of slopes was tested using interaction terms and if no significant interactions were found, an analysis of covariance was performed.

Residual plots were examined for equality of variance and normality of the data. For data where equality of variance was not satisfied, the data were log transformed. Analysis of covariance (ANCOVA) was followed by Tukey's multiple comparison test ($\alpha=0.05$). All statistical tests were deemed acceptable, as the residuals were found to be independent of the model, and normal in distribution (Sokal & Rohlf, 1995).

3.3 RESULTS

Mortalities were low ($4/320 = .0125\%$) and were not analysed (Table 3.1). Weight (g), standard length (cm) and body depth (cm) increased over the course of the

study (Table 3.1 and Figures 3.1 a, b, c). A nested ANOVA showed no significant differences (ie. no tank effect) between the replicates of treatments for either weight ($df=4$; $F=.71$; $p=0.583$), standard length ($df=4$; $F=1.11$; $p=0.351$) or body depth ($df=4$; $F=.66$; $p=0.620$) and subsequently data were pooled.

Overall data shows that photoperiod had no significant effect on weight ($df=3$; $F=0.61$; $p=0.611$), standard length ($df=3$; $F=1.22$; $p=0.300$), or body depth ($df=3$; $F=1.78$; $p=0.150$) of juvenile yellowtail flounder (Tables 3.1 and 3.2). There were no significant differences found among treatments for specific growth rates (SGR- Figure 3.2) ($df=3$; $F=0.31$; $p=0.818$) and / or gross food conversion ratios (GFCR's- Figure 3.3) ($df=3$; $F=0.15$; $p=0.922$).

Table 3.1: Mean initial weight (g \pm SE), mean final weight (g \pm SE), initial bottom coverage (%), final bottom coverage (%), initial condition (K) factor, final condition (K) factor, specific growth rate (% per day SGR), gross food conversion ratios (GFCR) and survival (%) for 1' yellowtail flounder under photoperiods of 24L:0D, 18L:6D, 12L:12D and ambient (which was adjusted every week to our latitude beginning September, 30) which were fed a ration of 2% body weight $\cdot d^{-1}$ (bw $\cdot d^{-1}$) which equalled satiation, (N= 40 per treatment).

Photo-Period Group	Mean Initial Weight (g)	Mean Final Weight (g)	Initial Bottom Coverage (%)	Final Bottom Coverage (%)	Initial K-factor	Final K-factor	Specific Growth Rate (% per day)	Gross Food Conversion Ratio (GFCR)	Survival (%)
24L:0D	8.96 \pm .45	36.12 \pm 1.50	10	23	1.81	2.01	1.25	1.85	100
18L:6D	9.37 \pm .43	36.49 \pm 1.68	10	23	1.81	2.01	1.22	1.88	100
12L:12D	9.34 \pm .32	35.14 \pm 1.19	10	22	1.75	2.06	1.19	1.84	96
Ambient	9.34 \pm .51	35.59 \pm 1.63	10	22	1.88	2.14	1.20	1.89	99

*, Indicates a significant difference between the treatments ($P < 0.05$). (Tukey's multiple comparison test).

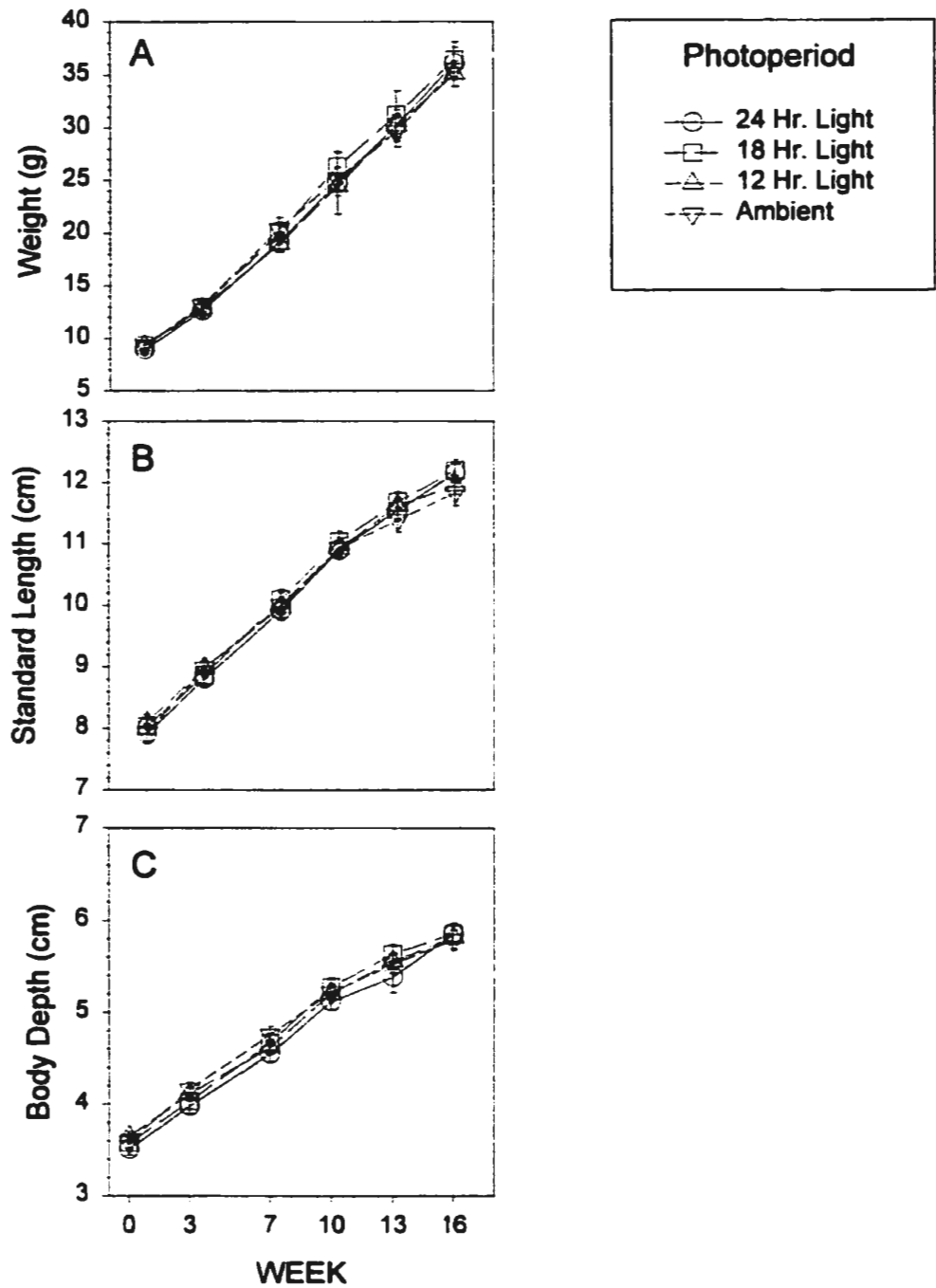


Figure 3.1 A) Mean weight (g), B) mean standard length (cm) and C) mean body depth (cm) of 1+ yellowtail flounder under photoperiods of 24L:0D, 18L:6D, 12L:12D and ambient. Vertical bars represent standard error. N= 40 per treatment.

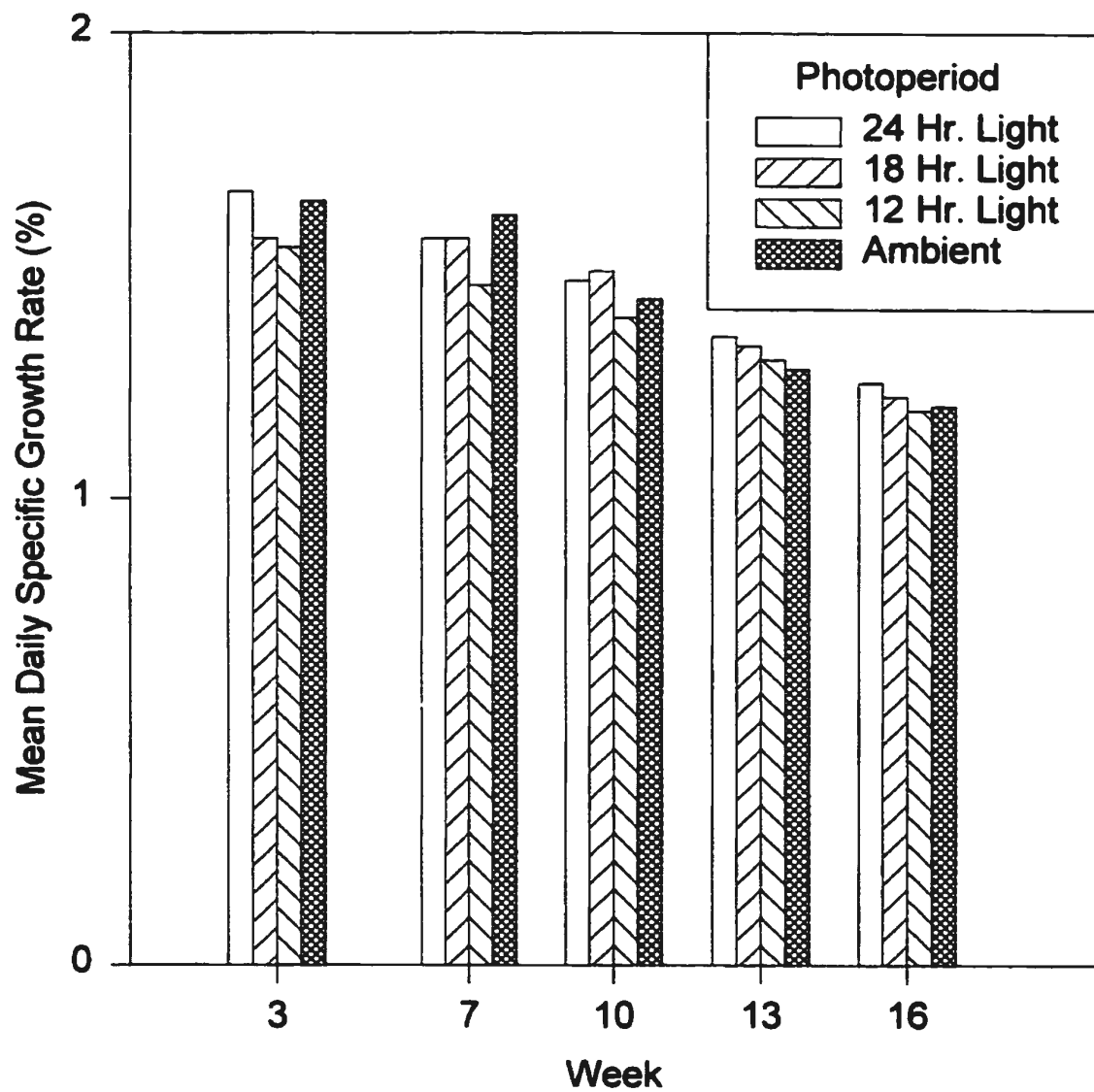


Figure 3.2 Mean daily specific growth rate of 1+ yellowtail flounder under photoperiods of 24L:0D, 18L:6D, 12L:12D and ambient.

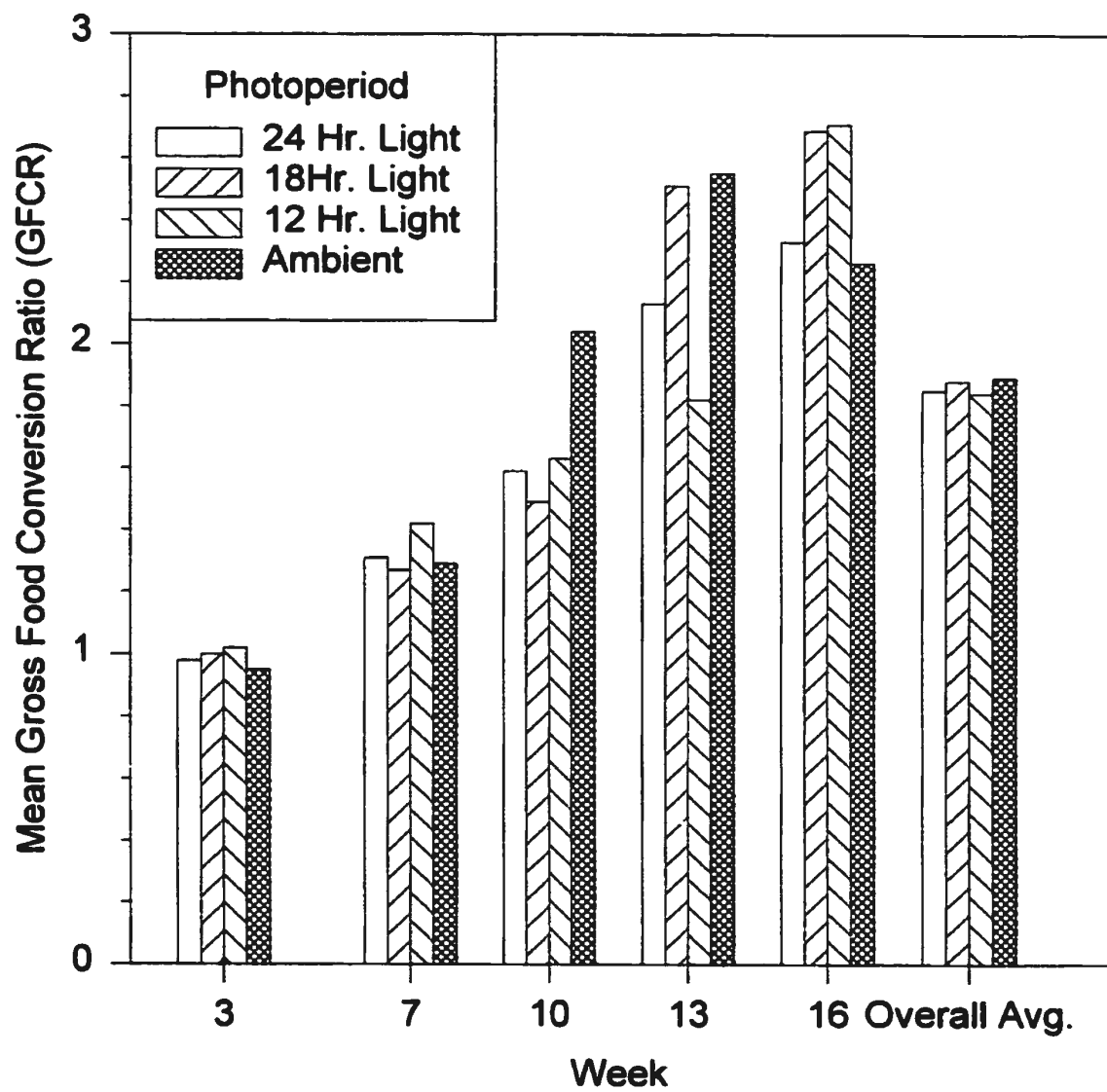


Figure 3.3 Mean gross food conversion ratios of 1+ yellowtail flounder under photoperiods of 24L:0D, 18L:6D, 12L:12D and ambient.

Table 3.2: Results of Tukey's Range Test for weight (WW), standard length (SL) and body depth (BD) for 1⁺ yellowtail flounder under photoperiods of 24L:0D, 18L:6D, 12L:12D and ambient (which was adjusted every week to our latitude beginning September, 30) which were fed a ration of 2% body weight·d⁻¹ (bw·d⁻¹) which equalled satiation, (N= 40 per treatment). Values have been log transformed for this table. Means with the same letter are not significantly different (p<0.05).

Dependent Variable	Treatment	Mean	N=	Tukey Grouping
Weight	18L:6D	24.7467	240	A
	Ambient	22.2339	240	A
	24L:0D	21.9895	240	A
	12L:12D	21.881	240	A
Standard Length	18L:6D	10.32208	240	A
	12L:12D	10.25813	240	A
	24L:0D	10.20604	240	A
	Ambient	10.17542	240	A
Body Depth	18L:6D	4.84167	240	A
	Ambient	4.84125	240	A
	12L:12D	4.82625	240	A
	24L:0D	4.75625	240	A

3.4 DISCUSSION

In this 16 week study photoperiod did not significantly affect the growth or survival of juvenile yellowtail flounder.

Increased growth of larvae has been demonstrated under longer photoperiod for several marine species, including sole, *Solea solea* (Fuchs, 1978), sea bass, *Dicentrarchus labrax* (Barahona-Fernandes, 1979), black porgy, *Mylio macrocephalus* (Kiyono &

Hirano, 1981), gilthead sea bream, *Sparus aurata* (Tandler & Helps, 1985), rabbitfish, *Siganus guttatus* (Duray & Kohno, 1988), barramundi, *Lates calcarifer* (Barlow *et al.*, 1995), Atlantic cod, *Gadus morhua* (Puvanendran & Brown, 1999) and yellowtail flounder, *Limanda ferruginea*, (Puvanendran, pers. comm.).

Photoperiod did not affect the juvenile growth in sole, *Solea solea* (Fuchs, 1978), black porgy, *Mylio macrocephalus* (Kiyono & Hirano, 1981), barramundi, *Lates calcarifer* (Barlow *et al.*, 1995), and Atlantic halibut, *Hippoglossus hippoglossus* (Hallaråker *et al.*, 1995). Growth was significantly increased in the juveniles of splitnose rockfish, *Sebastes diploproa* (Boehlert, 1981), Atlantic cod, *Gadus morhua* (Folkvord & Otterå, 1993), turbot, *Scophthalmus maximus*, (Imsland *et al.* 1995) and winter flounder, *Pleuronectes americanus*, (Casey & Litvak, unpublished data) when reared under extended photoperiods.

Increased photoperiod can influence growth of fish through physiological mechanisms such as increased hormone production (Brett, 1979; Meissel & Dodt, 1981; Bittman, 1985), which in turn can lead to improved food conversion efficiency (Collie & Stevens, 1985; Busacker *et al.*, 1990; Sun & Farmanfarmaian, 1992; Fine *et al.*, 1993; Peter & Marchant, 1995). Hence, increasing photoperiod has the potential of increasing growth rates without an increase in food consumption.

In experiments where fish are fed to satiation, increased photoperiods may cause higher growth rates through increased food consumption (Clarke *et al.*, 1978; Boehlert, 1981; Folkvord & Otterå, 1993; Imsland *et al.*, 1995). In my experiment, however, fish

were fed to satiation and no significant differences in growth were found. This suggests that when food is not limited, photoperiod still has no effect on growth.

The degree to which a particular species of fish responds in terms of growth and survival to changes in photoperiod seems to be largely influenced by their natural distribution. Freshwater or anadromous species generally show greater responses to photoperiod than do those found in the marine environment (Imslund *et al.*, 1995).

There are many factors which influence the growth of juvenile fish in hatchery/on-growing situations. Temperature, stocking density, light intensity, diet, and tank-type (raceway, etc.) are all factors which can affect growth and require investigation before optimal rearing environments for juvenile yellowtail flounder are determined.

A study looking at the effect of continuous feeding over the 24-hour light cycle may be useful in gathering growth data to determine the limitations of fish growth over time. Future research to test effects of photoperiod on sexual maturation may also be important for smaller flatfish species to determine if longer day lengths may suppress maturation. My experiment showed that photoperiod does not have a significant impact on the growth or survival of juvenile yellowtail flounder. Therefore longer photoperiods (18-24 daylight) are not necessary for optimal on-growing and suggest that yellowtail flounder juveniles can be cultured under ambient photoperiod, thereby eliminating the cost of artificial illumination.

CHAPTER 4

4.1 INTRODUCTION

Variation in growth rates and relative sizes attained by individuals of a cohort in a given space is a ubiquitous phenomenon (Ambeker & Doyle, 1990). One of the obvious differences between the natural environment and artificial environment is the high population densities at which hatchery fish are maintained (Fenderson & Carpenter, 1971). Increased stocking density results in stress which could lead to enhanced energy requirements causing reduced growth and food utilization (Hengsawat *et al.*, 1997). In hatcheries, it is routine to reduce the number of fish per rearing unit as the mean size of the fish increases. Often the fish from different batches are grouped together following grading to establish more homogenous size distribution, but it is not known what consequences this may have for subsequent growth performance (Jobling & Wandsvik, 1983).

Thus, there is a need to establish an “optimal stocking density” for cultured species in hatchery and on-growing situations. An important factor determining the economic feasibility of any aquaculture species is the maximum stocking density that can be maintained without substantial reduction in growth rate and survival (Björnsson, 1994). Research into stocking densities of juvenile flatfish during initial and final grow-out phases is limited. We need a way of predicting the growth rate to market size based on the juvenile growth rates at optimal stocking densities (Dambo & Rana, 1992).

Previous research dealing with stocking densities of salmonid species has dealt

primarily with social interaction, growth depression, spatial requirements, behavioural responses and growth variation (Fenderson & Carpenter, 1971; Refstie, 1977; Soderberg & Meade, 1987; Brown *et al.*, 1992b; Papst *et al.*, 1992). For flatfish species like turbot, *Scophthalmus maximus* L. (Martinez-Tapia & Fernández-Pato, 1991; Lyngstad, 1994; Klokseth & Øiestad 1999a), Olive flounder, *Paralichthys olivaceous* (Chang & Yoo, 1988, Chang *et al.*, 1995; Jeon *et al.*, 1993), Atlantic halibut, *Hippoglossus hippoglossus* L. (Björnsson, 1994; Klokseth & Øiestad 1999b), summer flounder, *Paralichthys dentatus* (King *et al.*, 1998) and winter flounder, *Pseudopleuronectes americanus* (Casey & Litvak, unpublished data) there is evidence that higher stocking densities affect growth.

Chang & Yoo (1988) used % bottom coverages for small (2.5 cm) fish up to 410% (4.1 times the bottom surface area) with flounder, *Paralichthys olivaceous* juveniles without negatively affecting growth. However, Björnsson (1994) on the other hand found that stocking density did have a negative affect on the growth rate of larger halibut (5-14 kg) when densities above 100% coverage of the tank bottom were used.

To date, few studies have demonstrated optimal stocking densities for juvenile flatfish under 10 g and none have examined 0+ juvenile yellowtail flounder stocking density. The objective of this experiment was to determine the optimal stocking densities for growth and survival of small juvenile 0+ yellowtail flounder.

4.2 MATERIALS AND METHODS

The experiment was carried out at the Ocean Sciences Centre (OSC.), Memorial

University of Newfoundland. Fish used in this experiment were reared from broodstock held at the OSC. Eggs were stripped from broodstock and all larvae were reared at the OSC.

Two weeks prior to the start of the experiment, fish were taken from holding tanks and transferred to experimental groups for acclimation. Three groups (with 2 replicates) were established with densities of (0.47, 0.95, 1.9 kg·m⁻² or 25, 50 and 100 fish per tank) with a % bottom coverage of 22.5, 45 and 90% respectively (refer to Appendix 1). Densities of (0.47, 0.95, 1.9 kg·m⁻²) were chosen and the results would create a baseline for future stocking densities. Fish were matched for weight (mean ± SE = 1.02 ± 0.05 g), standard length (mean ± SE = 3.96 ± 0.06 cm) and body depth (mean ± SE = 1.69 ± 0.03 cm) at the beginning of the experiment. The fish were placed in 0.26 m diameter black circular 13.5 litre tanks (22 cm water depth), provided with filtered salt water (mean ± SE = 7°, ± 0.3°C) at a rate of 0.5 L/min. exchange rate of (2.2 exchanges per hour). Growth measurements (weight, standard length and body depth) were taken at 14 day intervals on ten fish from each group (20 from each treatment). Fish were not fed on the day before measurements were taken.

Fish were fed a ration of 2% body weight·d⁻¹ (bw·d⁻¹). A formulated pellet of fry feed kyowa (C700-C1000 Biokyowa) was fed over a 30 to 40 min. period (apparent satiation) three times every second day (0900, 1500 and 2100 hours), and any uneaten food removed the following day. Biomass was calculated daily on achieved specific growth rates to ensure a 2% bw·d⁻¹ ration. Food was provided every second day to

minimize intraspecific variation in growth rates within groups of fish or to reduce hierarchy effects common among cultured species (Irwin *et al.*, 1997). Food ration selection and schedules was based on previous growth experiments conducted at the OSC (Chapter 2&3).

Light levels were kept at ~ 600 lux at the water surface and a photoperiod of 18L:6D with artificial dawn and dusk. Artificial dawn and dusk was maintained by low wattage lamps in the ceiling controlled by a timer. The experiment lasted for 10 weeks.

4.2.1 DATA ANALYSIS

Data on mean weight (g), standard length (cm), body depth (cm), specific growth rate (SGR), gross food conversion ratio (GFCR), coefficient of variance (CV %), Fulton's condition factor (K), stocking density or percent bottom coverage (kg·m⁻² or %) and survival were collected (see appendix I).

Data was analysed using SAS/STAT (SAS Institute, 1988). A nested analysis of variance (ANOVA) (Zar, 1982) was used to test for tank effects. A General Linear Model (GLM) determined if age or treatment influenced the growth parameters of juveniles under different treatments (stocking densities) and for each growth measurement. Homogeneity of slopes was tested using interaction terms and if no significant interactions were found, an analysis of covariance was performed.

Residual plots were examined for equality of variance and normality of the data. For data where equality of variance was not satisfied, the data were log transformed.

Analysis of covariance (ANCOVA) was followed by Tukey's multiple comparison test ($\alpha=0.05$). All statistical tests were deemed acceptable, as the residuals were found to be independent of the model, and normal in distribution (Sokal & Rohlf, 1995).

4.3 RESULTS

Mortality was low (10/350 or 3.5%) during the experiment. Mortality was highest in the treatment with the higher stocking densities (Table 4.1). Coefficients of variation (CV, %) among densities was minimal between treatments and remained fairly constant during the study (Figures 4.1 a, b, c).

A nested ANOVA showed no significant differences (ie. no tank effect) between the replicates of treatments for either weight (df=3; F=0.25; p=0.866), standard length (df=3; F=0.28; p=0.839), or body depth (df=3; F=0.36; p=0.780) and subsequently data were pooled. Overall data shows that stocking density had no significant effect on weight (df=2; F=1.21; p=0.301), standard length (df=2; F=3.25; p=0.040), or body depth (df=2; F=1.98; p=0.139) of juvenile yellowtail flounder.

Statistical analysis (ANCOVA) showed no significant difference between stocking densities of 25, 50 or 100 fish per tank (Table 4.2) with overall specific growth rates, overall gross food conversion ratios or survival. Even though not significant (SGR:df=2; F=3.36; p=0.1716) and (GFCR:df=2; F=5.02; p=0.1104), treatments held at lower and medium stocking densities had higher values of specific growth rate (SGR's), lower gross food conversion ratio values (GFCR) and higher survival than treatments held at highest densities (Table 4.1, Figures 4.2 a, b, c, Figure 4.3 and Figure 4.4).

Table 4.1 Mean initial weight (g \pm SE), mean final weight (g \pm SE), initial bottom coverage (%), final bottom coverage (%), initial condition (K) factor, final condition (K) factor, specific growth rate (% per day SGR), gross food conversion ratios (GFCR) and survival (%) for 0' yellowtail flounder with % bottom coverages of 22.5%, 45% and 90% per tank which were fed a ration of 2% body weight·d⁻¹ (bw·d⁻¹), (N= 20 per treatment).

Density	Mean Initial Weight (g)	Mean Final Weight (g)	Initial Bottom Coverage (%)	Final Bottom Coverage (%)	Initial K-factor	Final K-factor	Specific Growth Rate (% per day)	Gross Food Conversion Ratio (GFCR)	Survival (%)
25	1.00 \pm .08	2.33 \pm .19	22.5	39	1.63	1.72	1.21	1.54	98
50	1.01 \pm .07	2.33 \pm .17	45.0	78	1.71	1.79	1.20	1.55	100
100	0.98 \pm .10	1.93 \pm .17	90.0	131	1.61	1.81	0.91	2.25	96

***, Indicates a significant difference between the treatments (P<0.05). (Tukey's multiple comparison test).**

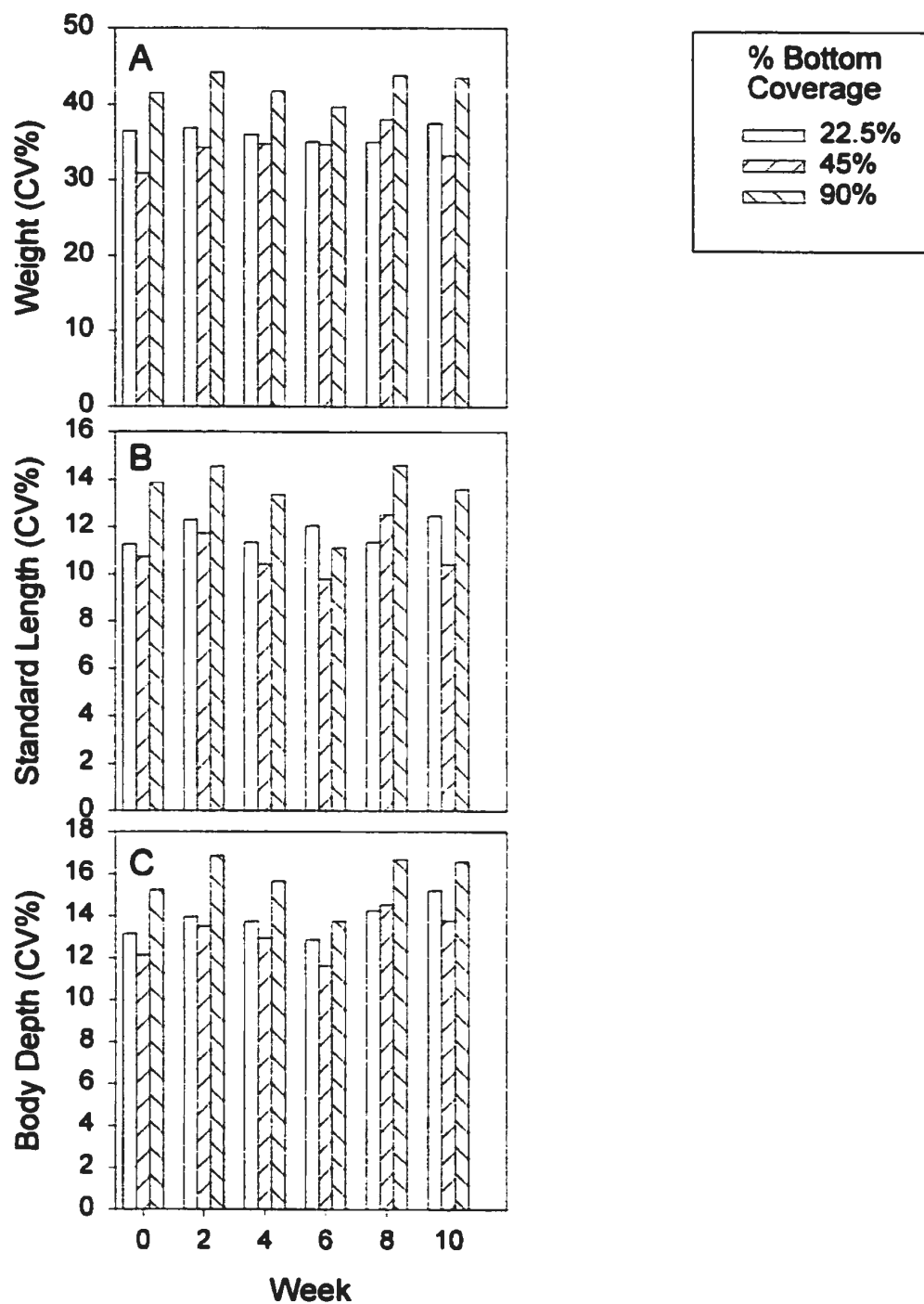


Figure 4.1 Mean coefficient of variation (%) for A) weight (g) B) standard length (cm) and C) body depth (cm) of 0+ yellowtail flounder with % bottom coverages of 22.5%, 45% and 90% per tank. N= 20 per treatment.

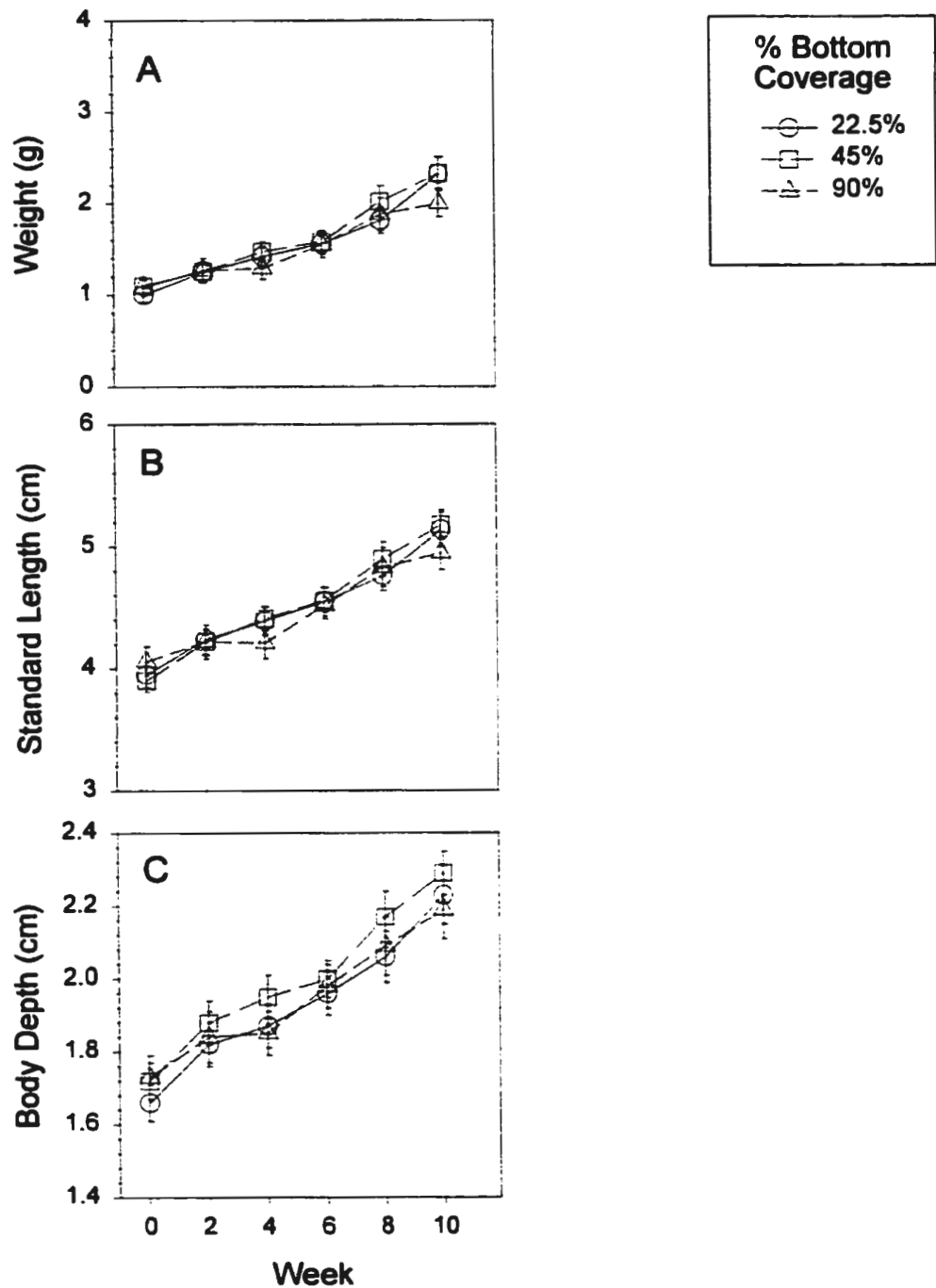


Figure 4.2 A) Mean weight (g), B) mean standard length (cm) and C) mean body depth (cm) of 0+ yellowtail flounder with % bottom coverages of 22.5%, 45% and 90% per tank. Vertical bars represent standard error. N= 20 per treatment.

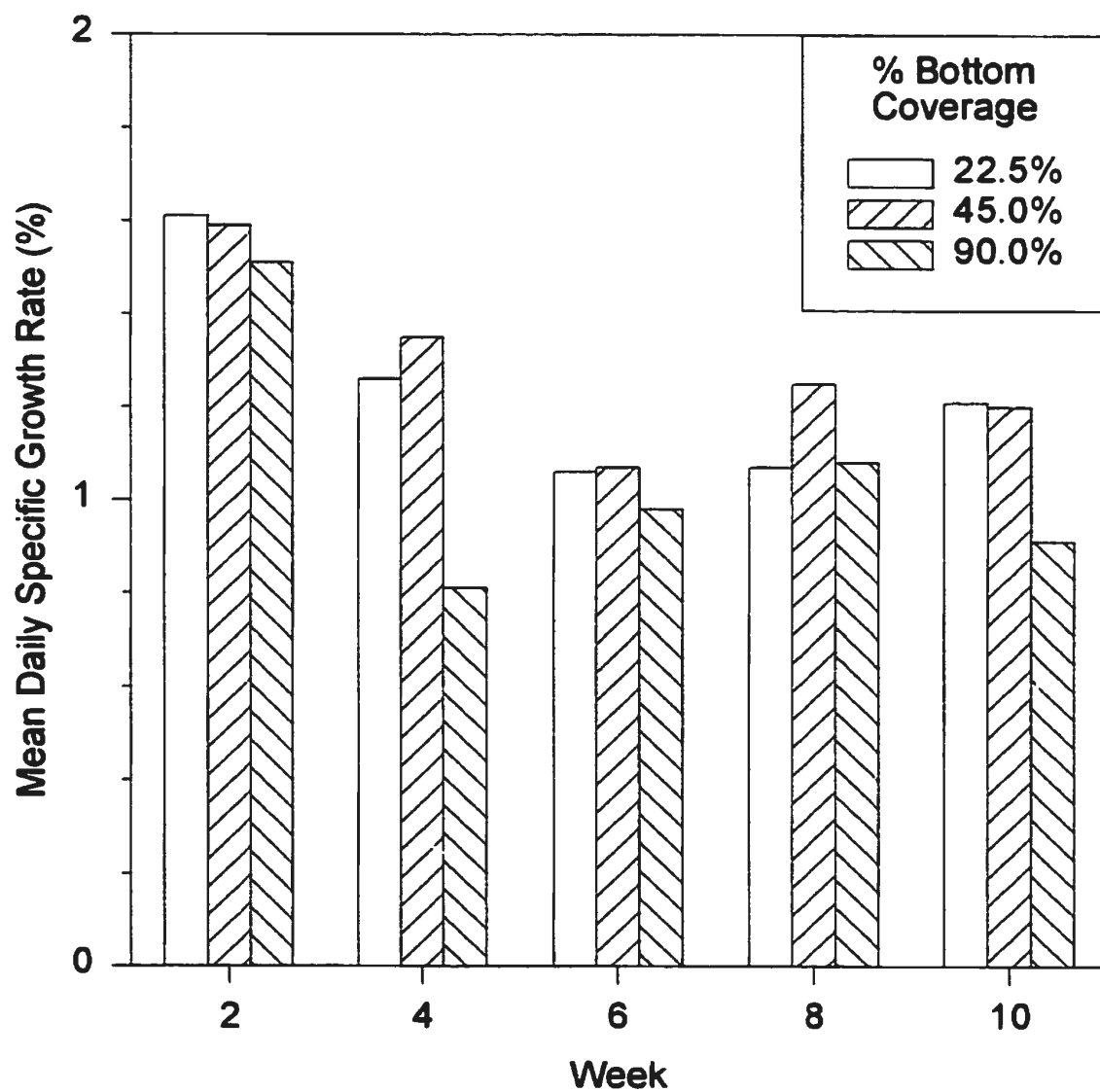


Figure 4.3 Mean daily specific growth rate of 0+ yellowtail flounder with % bottom coverages of 22.5%, 45% and 90% per tank.

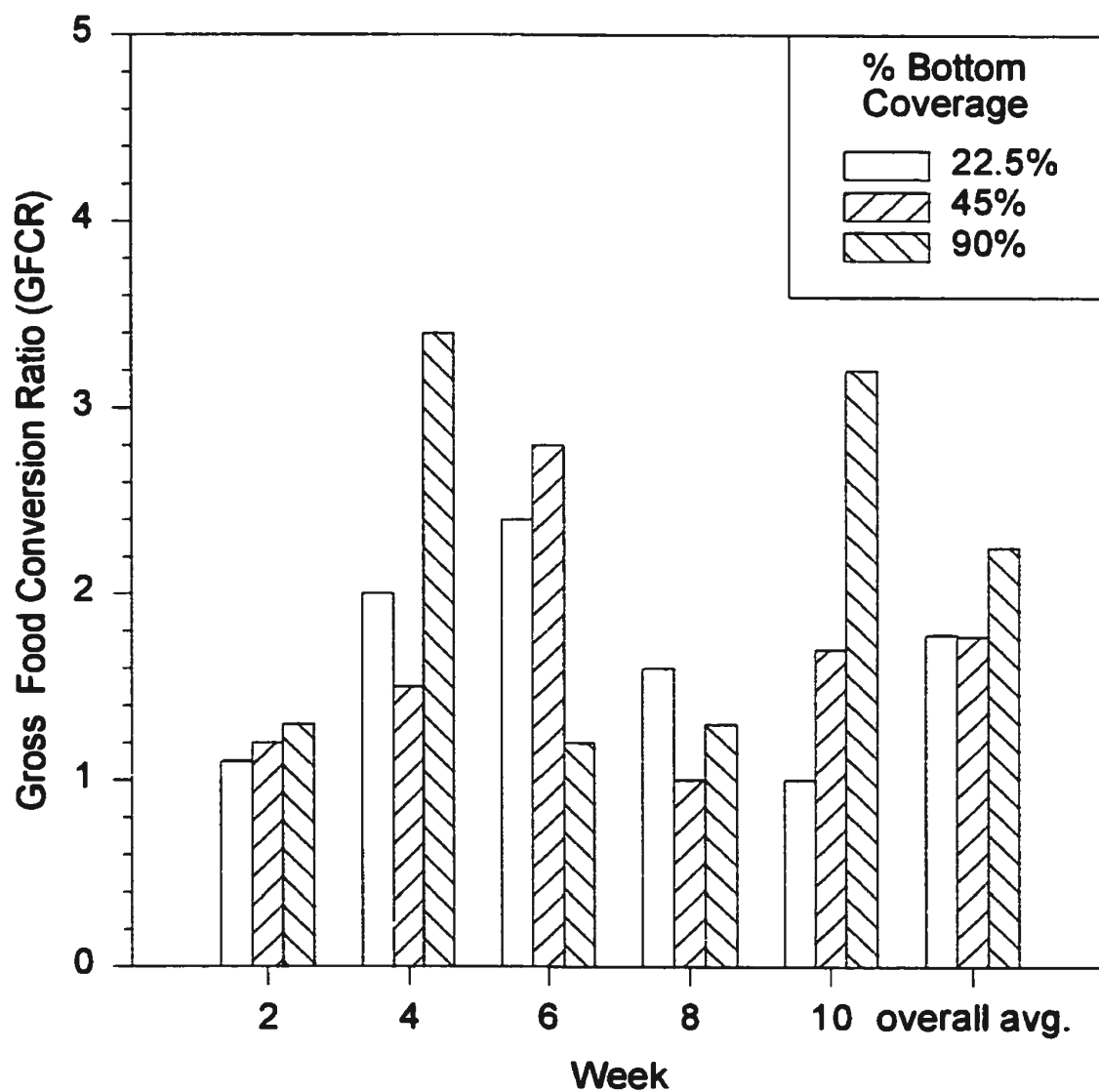


Figure 4.4 Mean gross food conversion ratios (GFCR) of 0+ yellowtail flounder with % bottom coverages of 22.5%, 45% and 90% per tank.

Table 4.2 Results of Tukey's Studentized Range Test for weight (WT), standard length (SL) and body depth (BD) for yellowtail flounder held under densities of 25, 50 and 100 per tank which were fed a ration of 2% bw·d⁻¹. (N= 20 per treatment). Values have been log transformed for this table. Means with the same letter are not significantly different (p<0.05).

Dependent Variable	Treatment	Mean	N=	Tukey's Grouping
Weight	50	4.52583	120	A
	25	4.50083	120	A
	100	4.4225	120	A
Standard Length	50	1.99917	120	A
	25	1.93083	120	A
	100	1.91667	120	A
Body Depth	50	1.60967	120	A
	25	1.56108	120	A
	100	1.47158	120	A

4.4 DISCUSSION

Results indicate that small, recently weaned yellowtail flounder stocked at initial densities of 0.47, 0.95, 1.9 kg·m⁻² (25, 50, 100 fish/tank) corresponding to 22.5, 45 and 90% coverage of the tank bottom by fish showed similar growth patterns during the 10 week study.

Fish stocked at the highest density (90% coverage) showed a trend towards lower growth than those in both of the other two treatments. Brown *et al.* (1992a), suggests that "growth rates for salmonoids is similar to that of other fish species in that growth

rates are higher at lower stocking densities, and as density increases, growth rates tend to decrease proportionally". Increased densities may lead to increased aggression which in turn may reduce growth rates. However, in this experiment growth was not reduced enough to warrant the reduction of the number of fish at the highest stocking density.

Fish density at the end of the 10 week experiment reached 1.10, 2.19, 3.39 kg·m⁻² or 39, 78 and 131% bottom coverage without impacting growth, which suggests that yellowtail can be stocked at densities greater than 100% coverage. King *et al.* (1998) showed that 0.7 g summer flounder could be stocked at densities of at least 200% coverage. Klokseth and Øiestad (1999a) showed that densities up to 210% coverage and growth rates of 4.5-11 SGR % are possible with 1-12 g turbot in raceway systems. Chang and Yoo (1988) used % bottom coverages for fish up to 410% with flounder, *Paralichthys olivaceous* juveniles without negatively affecting growth. Litvak (1999 Casey & Litvak, unpublished data) tested stocking densities of 50, 100 and 150% bottom coverage for winter flounder, *Pseudopleuronectes americanus* and found no difference in growth and survival among treatments. However, Björnsson (1994) found that stocking density affects growth rate of larger halibut (5-14 kg.) only above a certain threshold of 100% coverage of the tank bottom. It is important that to note that fish size may be an important factor in determining stocking density as well.

The high degree of homogeneity in the coefficients of variance with weight, length and body depth throughout this experiment suggests that all fish were given equal opportunity to feed and a 2% body weight·d⁻¹ (bw·d⁻¹) ration was adequate (Jobling,

1982). This is also supported by (Koebele, 1985) as cited by Dambo and Rana (1992) that if there is no size disproportional food acquisition, there will not be a size hierarchy effect. Once a size hierarchy within a population is established, the smaller fish are inhibited from feeding satisfactorily because of the physical presence of the larger fish (Dambo & Rana, 1992). Begon (1982) observed that smaller individuals perceive the population density as being much higher than it is, while larger individuals perceive the density as being lower than it is. Increased coefficients of variation when fish are reared in groups rather than in isolation have been observed in plaice, flounders and their hybrids (Purdom 1974) as cited by Dambo and Rana (1992). Understanding size variation between individual groups may be worth pursuing when raising flatfish at high stocking densities. Size grading may be an important factor in improving growth rates of hatchery raised fish.

Westers (1987) cited by Dambo and Rana (1992) suggested that under hatchery conditions, the condition factor averages about the same for groups of fish. As they grow the values often remains relatively constant during the hatchery life cycle (Dambo & Rana, 1992). There are many interesting relationships between growth parameters and stocking densities that can be addressed in future research.

In conclusion, results were positive (growth rates and survival) but I did not determine the upper limit of stocking densities which may have been one reason why there were no differences between treatments for yellowtail flounder. Additional experiments could be conducted to determine optimal stocking densities using small and large fish, grading times during production cycles, larger tanks, shelving, raceway systems and water

quality parameters as stocking density increases.

CHAPTER 5

SUMMARY, GENERAL DISCUSSION AND FUTURE RESEARCH

The experiments described in this thesis examined feeding and on-growing strategies for juvenile yellowtail flounder *Limanda ferruginea* (Storer).

Optimisation of feed efficiency is of utmost economic importance in aquaculture. Even with nutritionally adequate diets, much variation might be encountered in terms of feed efficiency, depending on feeding practices and schedules. Ration sizes should be adapted to nutrient, energy needs and growth rates. It is important to consider temperature, fish size, genotype, growth rate, nutrient and energy requirements for a given growth performance, dietary energy density, nutrient concentration, etc. The food ration experiments in this thesis showed that smaller yellowtail flounder proportionally require more food ($\text{body weight} \cdot \text{d}^{-1}$ ($\text{bw} \cdot \text{d}^{-1}$)) during early stages of the juvenile period during year one.

There are many different views on fish feeding practices: one viewpoint is to feed the fish to the point of satiation and let the recipient indicate when sufficient food energy has been taken in. It is known that fish will eat more food than required for maximal growth, provided of course that they are given a high-quality feed and that water quality is optimal. Best feed-to-gain ratios are often obtained when fish are fed at a rate below satiation. It is complicated and time consuming, but more economical and cleaner to feed below satiation, since nutrients consumed in excess of requirements (as will happen in satiation feeding) are simply excreted. Over-feeding is a waste of money and an

unnecessary burden on the environment.

The point at which fish reach maximum protein deposition needs to be studied. In some species of larger fish, protein deposition levels off but fat deposition continues to increase. Protein gain makes live weight gain not lipid gain. This is very hard to achieve in small fish which weigh much less and have a lower percentage of tissue and muscle mass. A better understanding of the relationship between growth and nutrient deposition is important for marine species (economic models) and in particular the development of new species where economics determines its potential. A decrease in feed efficiency isn't always an economic loss if you can get a little more growth and have more product to sell. In future research, it may be important to look at protein and energy deposition as a function of intake as opposed to live weight gain.

People also argue that there are benefits of feeding to satiation. My experiments have shown that feeding to satiation offers the advantage of more uniform growth due to reduced competition in the early stages of juvenile fish (0⁺ - 1⁺). Faster grow-out may help to improve food conversion ratios (FCR's) further by lowering the percentage of energy allocated to daily metabolism. Faster grow-out also improves returns on capital investments as stocks can be turned over more quickly. Feed tables may often be tabulated to under-feed the fish in an attempt to avoid feed wastage and to minimize labour. A fixed ration or percent of body weight·d⁻¹ (bw·d⁻¹) feed amount used in my experiments have provided a baseline for feeding practices of yellowtail flounder and other small flatfish species.

To manually feed fish to satiation (or almost to satiation) requires an increase in the amount of work and / or labour. Today, automatic feed controllers are available. These provide all the benefits of feeding to satiation and minimize the associated risks of environmental impacts (feed wastage) and operating expenses (feed and labour). These feed instruments can detect satiation (or non feeding) by sensing the beginning of feed wastage in the effluent lines. They are designed for either on-shore culture systems with short solid retention times or cage systems. In addition to the economic benefits for commercial growers, this controller reduces variables for researchers conducting feed trials and other aquaculture research. These systems have data flow analysis components which provides instant feedback on fish behaviour, plus data is analyzed by the software.

Recognition of the important balance between energy and protein in diets is critical. The correct balance provides for maximum protein consumption and consequently the delivery of as high concentrations of amino acids as can be used to support the potential growth rate of fish. Advances can only be made from a baseline of precise information. This information has to be gathered from fish farmers and researchers and then incorporated back into diet formulation.

The choice of feed type and feeding regime are significant factors in waste pollution. Once rations are determined, the feeding frequency is important to minimize wastage. Proper feeding practices lead to lower FCR's which are important in production models and estimates. Gastric evacuation times at different temperatures may be worth researching as well to improve FCR's and in turn growth. In general, it seems better to

provide more feed less often, than small amounts at regular intervals. Understanding and utilizing good feed management is a critical component of being a successful fish farmer.

The photoperiod experiment showed that for juvenile yellowtail flounder the most cost-effective approach is to provide a simulated natural photoperiod. Extended daylight did not negatively affect the growth of juveniles, but did not increase growth either. Therefore, there is no advantage in keeping juvenile yellowtail flounder under extended light regimes.

It appears that some fish have distinct feeding patterns. They consume feed throughout the day (opportunistic) but have distinct peaks in food intake at dawn and dusk (crepuscular). Very rarely did the fish feed in total darkness when food was left over from earlier feedings. It has been shown that juveniles exposed to long day-lengths may consume more food, but this energy is expended as non-productive energy associated with increased activity of the fish. This concept may be a worthwhile pursuit for future research with yellowtail flounder. My photoperiod experiment dealt primarily with feeding to apparent satiation twice daily during the light phase of the shortest photoperiod, and examining the effects of different photoperiods on growth. Feeding patterns should suit the biological rhythms of the species being cultured. If feed distribution occurs out of phase with such spontaneous feeding rhythms, some decrease in feed efficiency is expected.

The final chapter in my thesis examined the effects of three different stocking densities on the growth performance and survival of 0+ juvenile yellowtail flounder. No

significant differences in growth or survival between juveniles were found under the different stocking densities. Results were positive but did not indicate the upper limit of stocking densities. Yellowtail flounder stocked at low densities usually lie side by side on the bottom overlapping each other with a portion of their bodies forming a dense patch in one area of the tank until densities increase so that the fish cover all parts of the bottom. Threshold levels for stocking densities may vary for different flatfish species or size of fish. Larger flatfish may overlap, but do not smother or deteriorate water quality in the same manner as smaller flatfish. If the oxygen concentration drops below a certain point, fish will have to leave the bottom and search for better oxygenated sea water. This in turn will increase the swimming activity of fish within a system. I did not examine this hypothesis but it may be included in future behavioural studies.

Lower growth and higher gross food conversion ratios were seen in tanks with the highest stocking densities. Proportionally more of the food consumed may have been used for metabolism than for growth.

Stocking densities used by fish farmers may be higher than those reported in the scientific literature, since they try to maximize space and increase profits. However, the fish farmer has to find a threshold between stocking density and water quality that has no negative effects on growth rates. The optimal level will depend on capital costs of the fish farm per unit of rearing area, availability of the species, changes in market prices and trends with size of fish being raised.

As outlined in my thesis, feeding and on-growing strategies are very important.

The experiments outlined in my thesis will serve as a foundation for rearing of small juvenile flatfish. Yellowtail flounder over the past five years has been targeted as a small flatfish for commercial production. My experiments along with many others have all helped in putting together a model (refer to Appendix 1) for yellowtail flounder rearing from egg/larvae to juvenile on-growing. Research and development in aquaculture of this relatively newly utilized species is an important component in exploring the viability of its market potential and bringing it to full commercialization. Further research into the optimization of on-growing diets, particularly in relation to their cost effectiveness may be useful. Feeding strategies and stock management practices on a larger scale may also be useful. The technology transfer and adaption process from this species to others have given us the ability to diversify this ever changing and challenging world of aquaculture. My experimental results will help bring this species closer to pilot and perhaps full scale commercial production.

Yellowtail Production Model (egg/larvae)

Age (d)	0	6/0	4	8	12	16	20	24	28	32	36	40	44
Life History	I<-Egg->	I<-----Yolk-Sac----->								Begin			
			I<-----First Feeding----->							I<-----Metamorphosis----->			
Egg (diam.)/ Larval size (Standard length, - mm)	0.8-0.9	2.9	4		5		7		8.5		10.5		13.5
Temperature (°C)		I<--10-->							12				
Tank System	I<250 L>	I<-----3000 L----->											
	(Conical Incubator)							Circular Tank/Raceway System					
Water Exchange (L/min)	I<-3.0 L/min->	I<-----1.5 L/min----->								I<-----4-5 L/min----->			
Light Intensity (lux)	I<600 Lux>	I<-----2400 Lux----->										I<-200 Lux->	
Photoperiod (L:D)	I<-----24L:0D----->												
Prey Type/s			I<-----Enriched Rotifer----->										
								I<Nauplii>	I<--Enriched Artemia----->				
Prey Densities (L ⁻¹)			I<-----8000----->					I<-----2000----->					
Green water (day ⁻¹)		I<-----10L----->						I<-----30L----->					
Number of Feedings (Live Food) (d ⁻¹)		I<-----3----->						I<-----2----->					
Weaning												Begins I<--300 um-->	
Stocking Density (L ⁻¹)			I<--10-30 larvae/Litre-->										

Yellowtail Production Model (Juvenile)

Age (months)	2	3	4	6	8	10	12	14	16	18	20	24
Life History	I<End of Metamorphosis>II<-----Juvenile----->											
Larval size (Standard Length - cm.)	2.0			7.0		11.0		13.0		16.0		20.0
Temperature (°C)	I<-----8 - 12----->											
Tank System	I<-----3000 L-----> Shallow Tank &/or Raceway											
Water Exchange (L/min)	I<-4-5L/min->I<-----15 L/min----->I<-----20-30 L/min----->											
Light Intensity (lux)	I<..1200 Lux-->I<-600 Lux->I<-----50-200 Lux----->											
Photoperiod (L:D)	I<-24L:0D->I<-18L:6D->I<-Min. Natural Photoperiod (6-8 hrs.)----->											
Prey Type/s	Enriched Artemia											
Prey Densities (L ⁻¹)	I<-200/L->I											
Green water (day ⁻¹)	I<-10L->I											
Number of Feedings (Live Food) (d ⁻¹)	I<-2->I											
Weaning	I<-500-750 um-->I<-1.0-1.5 mm-->I<-2.0-2.5mm-->I<-3.0-4.0 mm----->											
Ration (bw*d ⁻¹)	I<-3-4%-->I<-2.0%-->I<-1.0-1.5%----->											
Stocking Density	I<-100-200% Bottom Coverage----->											

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Appendix 1

DATA ANALYSIS

The data collected were analysed for calculation of average mean weight, length, body depth, specific growth rates, gross food conversion ratio, coefficients of variation, condition factor, stocking density or % bottom coverage for fish and survival rates (refer to appendix 1).

- 1) Mean weight = the average weight of fish at t days for each treatment.
- 2) Mean length = the average standard length of fish at t days for each treatment.
- 3) Mean body depth = the average body depth was taken from the base of the dorsal fin to the base of the anal fin of fish at t days for each treatment.
- 4) Specific growth rate = the specific growth rates (SGR's) were calculated using the individual weight measurements as follows:

$$\text{SGR}(\%/ \text{day}) = \{(\ln W_2 - \ln W_1) / t_2 - t_1\} \times 100$$

Where SGR (% / day) is the overall specific growth rate in body weight per day.

W_1 = Initial juvenile weight (g) at time t_1 for each sample.

W_2 = Final juvenile weight (g) at time t_2 for each sample.

t = Time in days.

- 5) Gross Food Conversion Ratio = the amount of food fed per day divided by the weight gain.

$$\text{GFCR} = F/G$$

F = Weight of food fed

G = Weight gain

6) Coefficient of variation = the term applied to standard deviation when it is expressed as a percentage of the sample means. It is a value to indicate the size variation within the population.

CV(%) = 100 x (SD/mean weight, length or body depth) for each treatment.

Where \bar{x} = sample mean weight, length or body depth.

s = standard deviation of mean weight, length or body depth.

7) Condition factor = Fulton's condition factor (Ricker, 1975) was calculated from:

$$K = 100 W L^{-3}$$

Where W and L represent wet body weight (g) and length (cm), respectively.

8) a) Stocking density -- **Density = kg · m⁻²**

#fish · weight (g) / area

ie: Initial stocking density for ration experiment 2 (Chapter 2) with 100 fish per treatment.

$$100 \text{ fish} \times 7.40 \text{ g} / 5024 \text{ cm}^2 = 740 \text{ g} / .5024 \text{ m}^2 = .740 \text{ kg} / .5024 \text{ m}^2 \\ = 1.47 \text{ kg} \cdot \text{m}^{-2}$$

b) % bottom coverage for fish

$$\text{Area} = K(\text{constant}) \cdot \text{length}^{-2} \text{ or } K = \text{area} / \text{length}^{-2}$$

K=0.28 for yellowtail flounder juveniles

area of tank / area of one fish · # fish in tank

ie: Tank size for ration experiment 2 (Chapter 2) = 5024 cm²

$$1 \text{ fish} = 15.07 \text{ cm}^2 \cdot 100 \text{ fish} = 1507 \text{ cm}^2$$

$$\% \text{ coverage} = 5024 / 1507 = 33.34\% \text{ bottom coverage}$$

c) This is another formula that may be used as well to calculate the surface area in cm² of an individual flatfish.

$$\text{Area} = 5.75 \cdot \text{wt}^{0.65}$$

9) **Survival Rate = this was calculated as $N_t/N_o \times 100$**

Where:

N_t = is the total number of fish at the end of experiment.

N_o = is the initial number of fish at the start of experiment.

